VOLUME 78 *December 1958* NUMBER 6

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS

Tracheal Fenestration as a New Method for the Therapeutic Management of Chronic Pulmonary Diseases and for the Experimental Exploration of the Bronchial Tree. E. E. Rockey, S. A. Thompson, I. G. Epstein, Edward Wesserman, and K. J. Ahn ... 815
Pneumonectomy in the Treatment of Pulmonary Tuberculosis. T. W. Shields, W. M. Lees, and R. T. Fox ... 822
Seven- to Eleven-Year Follow-up Study of the Results of Primary Thoracoplasty for Pulmonary Tuberculosis. The Relationship of Certain Variables of the Disease to Outcome. Horace H. Zinneman and William B. Tucker ... 832
An Analysis of Preoperative Bronchial Biopsy in One Hundred Patients with Pulmonary Tuberculosis. Neil C. Andrews, Clarence I. Britt, and Philip C. Pratt ... 839
Experimental Pulmonary Emphysema. Production of Emphysematous Bullae in the Rabbit by Infection with Tuberculosis. John W. Bell ... 848
The Stability of the Tuberculin Reaction. Solomon Kunofsky and Julius Katz ... 862
A Five-Year Pilot Study of Tuberculin Testing in Honolulu Schools. Progress Report of the First Two Years of Observation. Robert H. Marks, George Tokuyama, and Agnes Peterson ... 871
Chemical Analysis of the Role of Humoral Factors in Native and Acquired Resistance to Tuberculosis. Shunsaku Oshima, Yutaka Fujita, Atsuko Takeuka, Michiro Nakajima, and Shusuke Tsuji ... 884
Comparison of Toleration, Absorption, and Clinical Efficacy of Several Para-Aminosalicylic Acid Preparations in Three Hundred Tuberculous Patients. Sumner S. Cohen, Wen Y. Yue, and Shih H. Tsai ... 899
Case Reports:
Pulmonary Alveolar Proteinosis. A Progressive, Diffuse, Fatal Pulmonary Disease. Coyt R. Payseur, Benjamin E. Konwaler, and Leroy Hyde ... 906
Papilloma of the Bronchus. S. Sochocky ... 910
Notes:
Preliminary Observations on the Development of Atypical (Chromogenic) Variants of the H37Rv Strain of *M. Tuberculosis* Under the Influence of Streptomycin and Isoniazid *in Vitro*. M. S. Tarshis ... 921
Cycloserine Combined with Pyrazinamide in the Treatment of Pulmonary Tuberculosis. E. Toguri and R. J. Atwell ... 927
Zone Electrophoresis in Starch Gels. A Report on Smithies Method in Normal Adults and in Patients with Tuberculosis. R. N. Johnston and I. Steven ... 932
The Cultivation of the Bacille-Calmette-Guerin Strain of *M. Tuberculosis* (BCG). T. F. Lenert *et al.* ... 934
The Biologic Activity of Crude Extracts of BCG. G. L. Hobby *et al.* ... 939
Comparison of Isoniazid Concentrations in the Blood of People of Japanese and European Descent. Therapeutic and Genetic Implications. H. W. Harris *et al.* ... 944
Correspondence: On the Mechanism of Increasing the Bacteriotropic Potencies of Isoniazid in the Presence of Para-Aminobenzoic Acid (PABA). Eugen Kubala ... 949
Books ... 952
American Trudeau Society:
The Award of the Trudeau Medal for 1958 ... 957
ATS Formula for Determining Irregular Discharge Rates ... 959
Notices ... 960
Index of Authors ... 961
Index of Subjects ... 963
Index of Abstracts ... 153

Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

Copyright 1958, by the National Tuberculosis Association

PRINTED IN U. S. A.

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY

Medical Section of National Tuberculosis Association

EDITOR
WALSH McDERMOTT
New York, N. Y.

CONSULTING EDITOR
ESMOND R. LONG
Pedlar Mills, Va.

ASSISTANT TO THE EDITOR
Helen W. Miller

EDITOR OF ABSTRACTS
EDWIN D. KILBOURNE
New York, N. Y.

EDITORIAL BOARD

W. E. Adams, M.D.
Chicago, Ill.
William R. Barclay, M.D.
Chicago, Ill.
David T. Carr, M.D.
Rochester, Minn.
Aaron D. Chaves, M.D.
New York, N. Y.
Mrs. Shirley H. Forster
Washington, D. C.
Edward A. Gaensler, M.D.
Brookline, Mass.
Max B. Lurie, M.D.
Philadelphia, Pa.
Gardner Middlebrook, M.D.
Denver, Colo.
Paul C. Samson, M.D.
Lafayette, Calif.
Leon H. Schmidt, Ph.D.
Cincinnati, Ohio
George W. Wright, M.D.
Cleveland, Ohio
Robert L. Yeager, M.D.
Pomona, N. Y.

BUSINESS MANAGER
HENRY W. STEVENS

NOTICE TO SUBSCRIBERS AND CONTRIBUTORS

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES is published by the National Tuberculosis Association and issued monthly about the first of the month. The REVIEW is the official journal of the American Trudeau Society. A volume includes six numbers and begins with the January and July numbers.

Membership: All physicians and surgeons in the United States, Canada, and other countries who are interested in pulmonary and other thoracic diseases may apply for membership in the American Trudeau Society. The dues for active membership in the Society are $15* per year, which includes membership in the National Tuberculosis Association, copies of the Society's News Letter, and a subscription to the REVIEW and to the monthly BULLETIN of the National Tuberculosis Association.

Subscriptions: The subscription price of the REVIEW is $12* for the calendar year. Checks should be made payable to the National Tuberculosis Association. The price of single copies is $1.50* postpaid.

Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis, nontuberculous pulmonary diseases, and related subjects of interest.

MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1955 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title, and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

The publishers will not be responsible for manuscripts, illustrations, etc., lost in transit. In order to save expense for authors' corrections in proof, manuscripts should be carefully revised by the author before submission.

Reprints: Fifty reprints without covers of articles will be furnished to authors free of charge when requested in advance. Additional reprints may be ordered.

Advertising: Rates will be furnished by THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES, 1790 Broadway, New York 19, N. Y., on request.

The Publishers reserve the right to decline any advertising submitted. Acceptance of an advertisement does not constitute official endorsement of the product advertised.

* Effective January 1, 1958, membership dues will be $18.00 per year and the subscription price will be $15.00 for the calendar year. The price of single copies will be $2.00 postpaid.

Second-class postage paid at Baltimore, Md. Acceptance of mailing at a special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 2, 1918.

Emphasizing the *PREOPERATIVE* use of

Adrenosem®* SALICYLATE

(Brand of carbazochrome salicylate)

TO CONTROL OOZING AND BLEEDING

FOR THE CHILD PATIENT BEFORE AND AFTER ADENOIDECTOMY AND TONSILLECTOMY:

"1. Preoperative medication: For seven days before operation Adrenosem, in doses of 1 mg., was administered three times a day and a mixture of benzathine penicillin and triple sulfonamides, in doses of 1 teaspoonful three times a day."[1]

"On the day of operation, Adrenosem 5 mg. hypodermically is administered to minimize capillary bleeding."[2]

FOR ALL PATIENTS BEFORE LARYNGOLOGIC, BRONCHOSCOPIC AND ESOPHAGOLOGIC PROCEDURES:

"The routine preoperative use of Adrenosem in tonsil and adenoid surgery diminishes both the bleeding at the time of operation and the oozing in the immediate postoperative period."[3]

IN UROLOGICAL PROCEDURES:

"My own method is, after ensuring maximum haemostasis, to employ every known means of ensuring that clotting shall occur, viz. the use of (1) Oxycel, (2) adrenalin with procaine locally into prostatic capsule, (3) Adrenosem systemically . . ."[4]

IN PLASTIC SURGERY:

". . . this drug [Adrenosem Salicylate] is a valuable aid to plastic surgery in its ability to reduce blood loss and provide a clearer operative field."[5]

IN ORAL SURGERY:

"The difference in the control bleeding time and the bleeding time after the administration of 10 mg., intramuscularly or orally, of Adrenosem Salicylate was statistically significant."[6]

"No postoperative bleeding was noticed in those patients who had received Adrenosem Salicylate preoperatively."[7]

IN PATIENTS WITH BLEEDING DISTURBANCES:

"Treatment consisted of the administration of Adrenosem® Salicylate intramuscularly in quantities from 20 to 80 mg. over a period of four to eight days before operation."[8]

NON-TOXIC, NO CUMULATIVE EFFECT:

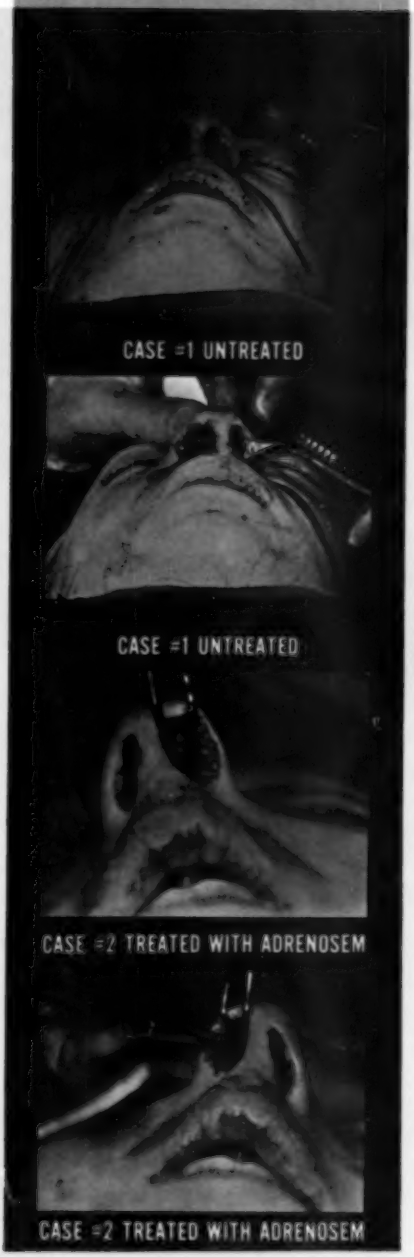
"Adrenosem Salicylate is non-toxic and has a high index of therapeutic safety. At the recommended dosage levels there are no contraindications. It has no cumulative effect. Patients treated for more than two years show no toxic effects attributable to the drug."[9]

*U.S. Pat. 2581850,2506294

THE S. E. MASSENGILL COMPANY

BRISTOL, TENNESSEE

NEW YORK • KANSAS CITY • SAN FRANCISCO



effective *preoperatively* in virtually every operative procedure

TO CONTROL OOZING AND BLEEDING

Adrenosem®

SALICYLATE

(Brand of carbazochrome salicylate)

The photographs to the left show the same rhinoplastic procedure on two patients—one untreated, one treated with Adrenosem.

Adrenosem is supplied: Ampuls, 1 cc., 5 mg.; Tablets, 1 and 2.5 mg.; Syrup, each 5 cc., 2.5 mg. Potency of all dosage forms stated in terms of the active ingredient, adrenochrome monosemicarbazone.

Write for literature describing the action and uses of Adrenosem Salicylate.

THE S. E. MASSENGILL COMPANY

BRISTOL, TENNESSEE • NEW YORK • KANSAS CITY • SAN FRANCISCO

1. Orzac, E.: Medical Care of the Child Patient Before and After Adenoidectomy and Tonsillectomy, N.Y. State J. Med. 56:886 (Mar., 1956).

2. Eraner, M.S. and Lerner, S.S.: M. Clin. North America 40:1749 (Nov., 1956).

3. Poole, J.C.: Adrenosem in the Control of Hemorrhage from the Nose and Throat, A.M.A. Arch. of Otolaryng. 61:450 (Apr., 1955).

4. Donohy, P.J.: The Care of the Prostatic Cavity, South African Med. 30:21 (Apr., 1956)

5. Brown, W.S.: The Use of Adrenosem Salicylate to Control Postoperative Bleeding in Plastic Surgery: Dermabrasion, Northwest Med. 57:470 (Apr., 1958)

6. Perkins, R.E.L.: A Clinical Investigation of Adrenochrome Monosemicarbazone Sodium Salicylate, Oral Surg., Oral Med., Oral Path. 10:230 (Mar., 1957).

7. Brode, A.B. and Chianese, T.C.: A Clinical Evaluation of Adrenosem Salicylate, Ann. of Dentistry 15:56 (Sept. 1956).

8. Zubiets C.B. and Escanoverino, R.B.: Adrenosem for the Prevention of Bleeding in Tonsillectomy, Am. Pract. 3:385 (Mar., 1957).

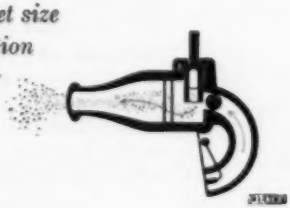
9. Riddle, A.C., Jr.: Adrenosem Salicylate: A Systemic Hemostat, Oral Surg., Oral Med., Oral Path. 6:617 (June, 1955).

NORISODRINE® Sulfate Powder in the **AEROHALOR®**
(ISOPROTERENOL SULFATE, ABBOTT) (POWDER INHALER, ABBOTT)

No matter what the grade of bronchial asthma, Norisodrine powder therapy stems the attack—bringing relief in mere seconds.

Just an inhalation or two of Norisodrine through the handy Aerohalor . . . that is all that is necessary to move the particles into the tracheo-bronchial tree where they will do the most good. The bronchial secretions remain not only undiminished but often increase.

Your asthmatics will appreciate the Aerohalor's discreet size and shape, which permits unobtrusive self-administration of Norisodrine. Too, this same compactness makes for convenient carrying in purse or pocket.



With dosage accurately adjusted, side effects are seldom experienced. Abbott

Meat...

and its fat-protein calorie ratio*

For every calorie of fat provided by the average American dietary only 0.3 calorie of protein is provided. The quality of this protein ranges from poor to excellent.

On the other hand, cooked meat as usually eaten supplies from three to five times this number of protein calories in relation to the calories derived from its fat. The protein of meat is invariably of high biologic quality.

	Calories from Fat	Calories from Protein
in the average American dietary	1	0.3
in beef rump roast	1	1.27
in beef flank steak	1	1.42
in lamb leg roast	1	1.55
in pork loin chop (lean portion only)	1	1.36
in cured ham	1	1.04
in veal cutlet (lean portion only)	1	1.40

The high protein yield of meat exceeds that from any other main dish food in man's diet. The amino acid composition of meat protein closely approaches the quantitative proportions needed for effective biosynthesis of human tissue.

Meat—the chief source of top-quality protein, B vitamins, and minerals in the American diet—is also an excellent vehicle for nutritionally valuable fat, the amount of which is readily controlled by trimming and by selection of cuts.

*Leverton, R.: The Fat, Protein, and Calorie Value of Cooked Meats. Proc. 9th Research Conf. sponsored by the Council on Research of the American Meat Institute, University of Chicago, March 1957.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

American Meat Institute
Main Office, Chicago...Members Throughout the United States

brings new dimensions to radiology of the heart and circulatory system and with far less radiation exposure

8" image (shown half-scale here) plus 30" target-to-table-top distance is big enough to visualize the whole average adult heart.

THE PICKER CARDIOLOGICAL X-RAY UNIT

A single comprehensive apparatus with which you can...

fluoroscope on an 8" amplifier tube
(The field is large enough to visualize the whole adult heart)

monitor continuously on closed-circuit TV
(Simultaneously with any number of remote TV screens)

record an examination on 16mm cinefilm
(At rates up to 60 frames-per-second)

fluoroscope in the large on a non-amplified 12" x 12" fluoroscopic screen
(By simply sliding the amplifier assembly out of the field)

get a 10" x 12" radiograph anytime during the examination
(Simply drop a loaded cassette into the well, expose, remove and go back to fluoroscopy)

Get the story from your local Picker representative or write
PICKER X-RAY CORPORATION
25 So. Broadway, White Plains, N. Y.

Picker x-ray

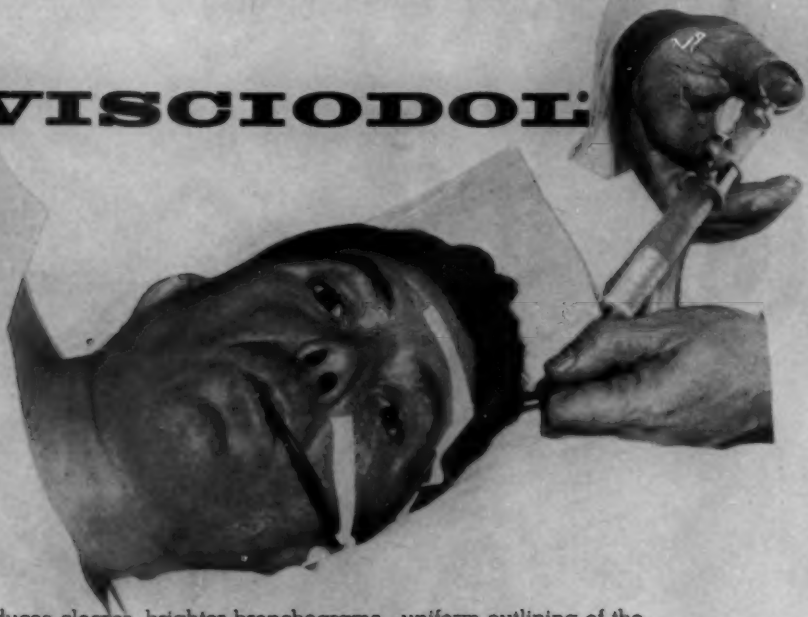
if it has to do with RADIATION it has to do with PICKER

now

a rapidly eliminated bronchographic medium...

less irritating—rarely penetrates the alveoli

VISCIODOL



- Produces clearer, brighter bronchograms—uniform outlining of the bronchial tree.
- Usually requires less anesthesia.
- Rapidly and completely eliminated—but retained long enough for adequate bronchographic studies.
- Thoroughly documented by reports on thousands of bronchograms.

SUPPLIED in 15 cc. vials. ***Stir well before use.***

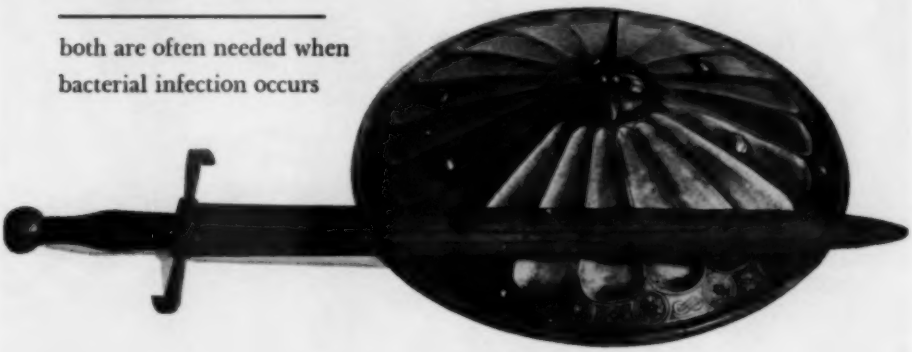
VISCIODOL® is the registered trademark of the well-tolerated LIPIODOL® (iodized oil) sulfanilamide suspension, manufactured under agreement with Andre Guerbet Laboratories, Paris, France.

E. FOUGERA & COMPANY, INC • NEW YORK 13, N.

▮ prompt, aggressive antibiotic action

▮ a reliable defense against monilial complications

both are often needed when bacterial infection occurs



for a direct strike at infection

Mysteclin-V contains tetracycline phosphate complex

It provides a direct strike at all tetracycline-susceptible organisms (most pathogenic bacteria, certain rickettsias, certain large viruses, and Endamoeba histolytica).

It provides the new chemical form of the world's most widely prescribed broad spectrum antibiotic.

It provides unsurpassed initial blood levels – higher and faster than older forms of tetracycline – for the most rapid transport of the antibiotic to the site of infection.

for protection against monilial complications

Mysteclin-V contains Mycostatin

It provides the antifungal antibiotic, first tested and clinically confirmed by Squibb, with specific action against Candida (Monilia) albicans.

It acts to prevent the monilial overgrowth which frequently occurs whenever tetracycline or any other broad spectrum antibiotic is used.

It protects your patient against antibiotic-induced intestinal moniliasis and its complications, including vaginal and anogenital moniliasis, even potentially fatal systemic moniliasis.

MYSTECLIN-V

Squibb Tetracycline Phosphate Complex (Sumycin) and Nystatin (Mycostatin)

Capsules *(250 mg./250,000 u.), bottles of 16 and 100.* Half-strength Capsules *(125 mg./125,000 u.), bottles of 16 and 100.* Suspension *(125 mg./125,000 u. per 5 cc.) 60 cc. bottles.* Pediatric Drops *(100 mg./100,000 u. per cc.). 10 cc. dropper bottles.*

SQUIBB *Squibb Quality – the Priceless Ingredient*

'MYSTECLIN'®, 'SUMYCIN'® AND 'MYCOSTATIN'® ARE SQUIBB TRADEMARKS

At any age

they welcome Ovaltine for extra nourishment

From pediatrics to geriatrics Ovaltine provides a rich source of the vitamins, minerals and other essential food elements required for the maintenance of a good nutritional state.

Ovaltine is a nourishing, well-tolerated beverage combining natural blandness with good taste. It produces a soothing and relaxing effect for the tense and nervous patient, particularly when taken at bedtime.

It is ideal for use where stimulating beverages should be avoided...ideal as nutritional fortification for patients on bland diets ...or to help maintain a satisfactory nutritional level during physiologic stress.

Three servings of Ovaltine and milk provide:

12 Vitamins		13 Minerals	
*Vitamin A	4000 I.U.	including Calcium, Phosphorus, Iron and Iodine	
*Vitamin D	420 I.U.	CARBOHYDRATE	65 Gm.
*Ascorbic acid	37.0 mg.	*PROTEIN	32 Gm.
*Thiamine	1.2 mg.	FAT	30 Gm.
*Riboflavin	2.0 mg.		
Pyridoxine	0.5 mg.		
Vitamin B_{12}	5.0 mcg.		
Pantothenic acid	3.0 mg.		
*Niacin	18.0 mg.		
Folic acid	0.05 mg.		
Choline	200 mg.		
Biotin	0.03 mg.		

*Nutrients for which daily dietary allowances are recommended by the National Research Council.

A jar of Ovaltine will be sent for your personal use on request.

Ovaltine® *when extra nourishment is desired*

The Wander Company, 105 W. Adams St., Chicago 3, Ill.

Clear the air

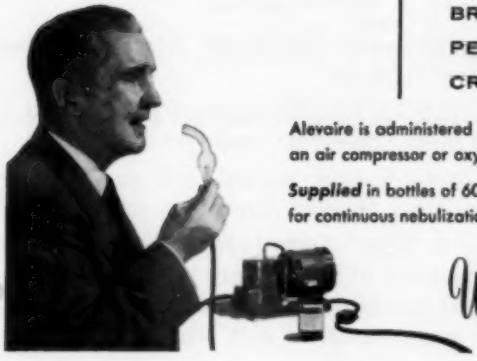
with ALEVAIRE®

inhalation therapy

WETS, THINS, LOOSENS PULMONARY SECRETIONS

Very useful in...

... BRONCHITIS
BRONCHIAL ASTHMA
BRONCHIECTASIS
PERTUSSIS
CROUP



Alevaire is administered by means of a nebulizer operated with an air compressor or oxygen.

Supplied in bottles of 60 cc. for intermittent and 500 cc. for continuous nebulization.

Winthrop LABORATORIES
NEW YORK 18, N. Y.

Alevaire, trademark reg. U.S. Pat. Off.

for the hospitalized tuberculous patient

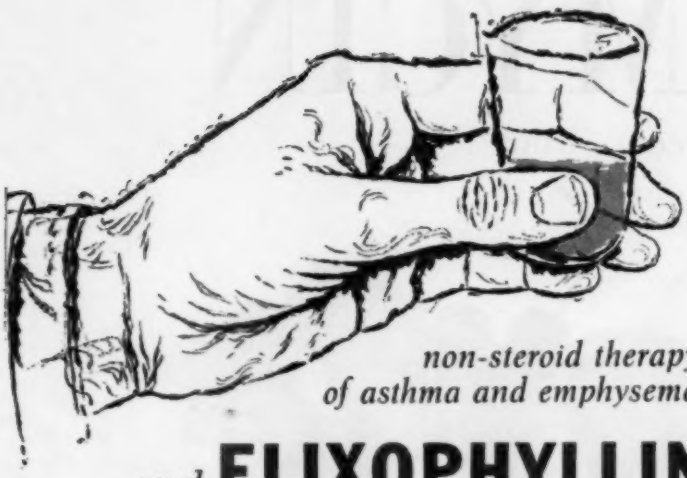
PYRAZINAMIDE

PYRAZINOIC ACID AMIDE

- *For the patient before surgery:* dramatic, potentially lifesaving tuberculostasis, to protect against spread of infection.
- *For extrapulmonary lesions:* draining sinuses, mucosal lesions, and lymphadenopathy—remarkable, rapid improvement or healing.
- *For relatively far-advanced fibrotic lesions:* partial clearing—in a larger percentage when acute, infiltrative—in a smaller percentage when chronic.

When the patient fails to respond to other chemotherapy: much short-term improvement. Fever, cough, and quantity of sputum are reduced and the patient gains weight, enjoys increased appetite and a sense of well-being. The antituberculotic effect is enhanced when combined with other drugs.

MSD **MERCK SHARP & DOHME**
DIVISION OF MERCK & CO., Inc., PHILADELPHIA 1, PA.



non-steroid therapy
of asthma and emphysema

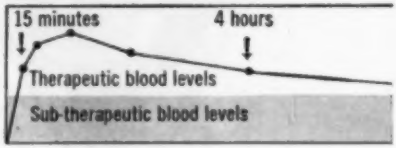
oral **ELIXOPHYLLIN**

Just as with I.V. aminophylline,* high theophylline blood levels *reached in minutes* – from a single dose.*

After absorption, theophylline is slowly eliminated. Therapeutic blood levels *endure for hours.**

This predictability of blood levels permits quite constant *therapeutic blood levels night and day,* providing relief of wheezing, dyspnea, cough, and protection against acute attacks for most patients.*

DOSAGE: *First two days:*
45 cc. *(three tbsp.)* on arising;
45 cc. *(three tbsp.)* on retiring;
45 cc. *(three tbsp.)* once midway between above doses *(about 3 P.M.)*



After two days of therapy the size of doses should be slightly decreased.
Each tablespoonful contains: theophylline 80 mg., alcohol 3 cc.
Prescription only—bottles of 16 fl. oz.

Sherman Laboratories
Detroit 11, Michigan

**Reprints of these studies on request.*

the flavor of fruit and a broad spectrum antibiotic

SUMYCIN

TETRACYCLINE

syrup / aqueous drops

new Sumycin Syrup

An aqueous tetracycline suspension, phosphate potentiated, containing 125 mg. tetracycline (hydrochloride equivalent) per 5 cc. teaspoonful.

60 cc. bottles

new Sumycin Aqueous Drops

An aqueous tetracycline suspension, phosphate potentiated, containing 100 mg. tetracycline (hydrochloride equivalent) per cc.

10 cc. bottles with the new, unbreakable 'FLEXIDOSE' DROPPER

. . . delicious aqueous suspensions to assure patient acceptance for effective tetracycline therapy . . . children especially will like their tasty fruit flavor

Also available:	Tetracycline phosphate complex equiv. to tetracycline HCl (mg.)	Packaging:
Sumycin Capsules	250 mg. per capsule	Bottles of 16 and 100
Sumycin Half Strength Capsules	125 mg. per capsule	Bottles of 16 and 100
Sumycin Intramuscular with Xylocaine*	250 mg. per vial	1 dose vials
Sumycin Intramuscular with Xylocaine*	100 mg. per vial	1 dose vials

SQUIBB 1858 1958 *Squibb Quality — the Priceless Ingredient*

'SUMYCIN'® AND 'FLEXIDOSE' ARE SQUIBB TRADEMARKS *T.M. @ ASTRA PHARMACEUTICAL PRODUCTS, INC. FOR LIDOCAINE.

ERRATUM

In table 2 of the article entitled "Pleural Biopsy in Diagnosis of Effusions" by Hill, Hensler, and Breckler, in the July, 1958, issue of the REVIEW (vol. 78, p. 8), the heading "Biopsy Positive for Acid-fast Bacilli" should have read "*Tuberculosis Demonstrated by Biopsy.*"

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 78 *December 1958* NUMBER 6

TRACHEAL FENESTRATION AS A NEW METHOD FOR THE THERAPEUTIC MANAGEMENT OF CHRONIC PULMONARY DISEASES AND FOR THE EXPERIMENTAL EXPLORATION OF THE BRONCHIAL TREE[1,2]

E. E. ROCKEY, S. A. THOMPSON, I. G. EPSTEIN, EDWARD WESSERMAN, AND K. J. AHN

(Received for publication July 16, 1958)

INTRODUCTION

Tracheal fenestration is a new method for the therapeutic management of chronic pulmonary diseases and for the experimental exploration of the tracheobronchial tree. It is a permanent skin-lined tracheocutaneous communication. The external opening of this skin tube is guarded by two doorlike valves which normally are in apposition and prevent the leakage of air or liquid. These valves may be manually opened and thus provide a short-cut entrance to the tracheobronchial tree for the purpose of aspiration of retained secretion and for medication.

EXPERIMENTAL EVOLUTION

Tracheal fenestration was developed experimentally on dogs in 1955 (1, 2). A step-by-step description of this operation is as follows:

A four-inch transverse incision is made over the anterior aspect of the lower third of the neck. The incision is extended in such a way that one skin flap is outlined above and one below the level of the initial transverse incision. Through the original transverse incision, the platysma is incised in a transverse direction to about an inch distal to the right and left of the midline. The trachea is then exposed by splitting the strap muscles. A 1.25 cm. length of two adjoining tracheal rings is excised with the underlying mucosa. The newly created tracheal window edges are covered by inverting the full thickness of the skin. This is accomplished by inverting the distal margin of each of the skin flaps and fixing them to the tracheal mucosa with interrupted mattress sutures of silk. The skin flaps are not dissected from their underlying base, but are held doubled by retaining sutures. The edges of the flaps are approximated with 000 silk sutures. This forms a right and left valve on each side of the tracheal window.

In the light of the difficulties encountered when the first tracheal fenestration was performed on a patient at Metropolitan Hospital, New York, on January 30,

[1] From The New York Medical College-Metropolitan Medical Center, New York, New York.

[2] Presented, in part, as a scientific exhibit at the New York Academy of Medicine Postgraduate Week, October 2 to 16, 1957.

1956, further animal experimentation was undertaken. These experiments led to the development of the present operation which is applied now to human beings.

In the present technique, the base of each of the opposing skin flaps is brought nearer to the midline. The distal margin of each of the opposing skin flaps covers two of the four tracheal window edges. The left skin flap covers the left and lower margins; and the right flap, the right and upper margins of the window (figure 1).

The addition of a temporary tracheotomy below the level of the fenestration has been found to be of added value (figure 2). The tracheotomy is utilized for the first two to three weeks, allowing time for the fenestration to heal. When this is accomplished, its clinical function starts and the tracheotomy below the fenestration is allowed to heal (figure 2).

Illustrative Cases

Case 1. F. V. was a 34-year-old Negro male with hopeless far advanced bilateral pulmonary tuberculosis. The left lung was completely destroyed; on the right side, there were numerous excavations occupying the entire upper half of the lung field and there were infiltrations in the right lower lung field (figure 3). The patient was febrile, emaciated, dyspneic, and cyanotic. He often had pulmonary hemorrhages, coughed constantly, and expectorated 400 to 500 ml. of sputum daily.

On January 30, 1956, a first-stage tracheal fenestration was performed. On April 5, 1956, the fenestration was completed. Tracheobronchial aspirations were applied as often as every ten to fifteen minutes. Once a day, on alternating sides, 0.5 gm. of cycloserine was instilled. The aspirations and medications led to a great reduction in the amount of bronchial secretion, eventually from 400 to 500 to 30 ml. per day, and the number of aspirations was reduced to three and four a day. The patient's breathing was easier; he was able to eat and sleep. Four months after the fenestration, he died of massive pulmonary hemorrhage.

This patient benefited a great deal from the repeated tracheobronchial aspirations and from the endotracheal instillation of drugs.

Case 2. J. C. was a 5½-year-old white male with the established diagnosis of fibrocystic disease of the pancreas with extensive pulmonary suppuration (figure 4). His cough was

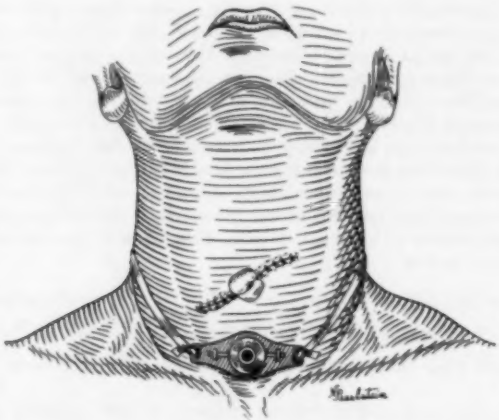


FIG. 1. The completed operation.

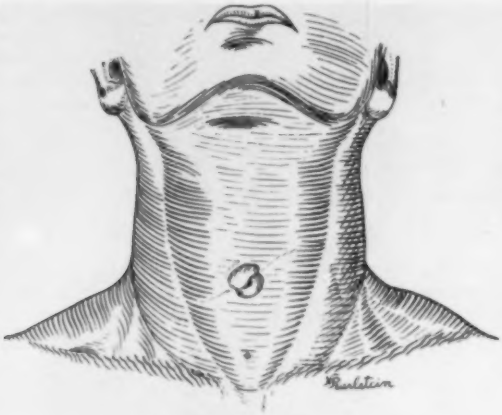


FIG. 2. The healed operation.

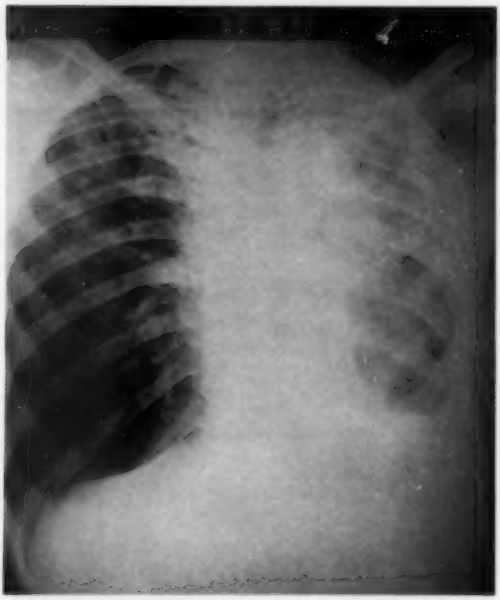


FIG. 3. Postero-anterior chest roentgenogram showing bilateral far advanced pulmonary tuberculosis. The left lung is completely destroyed. On the right side, there are numerous cavities occupying the upper half of the lung field.

excessive and, even considering his age, he expectorated a large amount of purulent sputum. Dyspnea, wheezing, and cyanosis were prominent symptoms. His clinical course was rapidly downhill.

On June 22, 1956, the patient was submitted to tracheal fenestration and a simultaneous temporary tracheotomy. Suctioning as often as every ten to fifteen minutes resulted in a

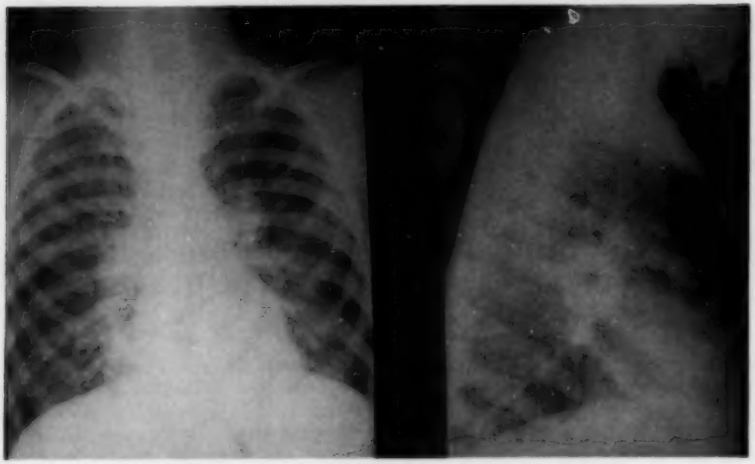


FIG. 4. Postero-anterior and lateral views of the chest showing extensive bilateral pulmonary infiltrations.

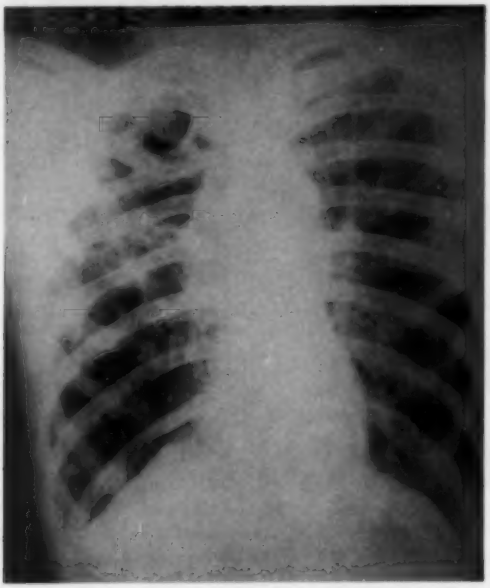


FIG. 5. Postero-anterior chest roentgenogram showing evidence of advanced emphysema and bilateral pulmonary infiltrations.

decrease of dyspnea, improved the ventilation, and decreased the respiratory rate. Large quantities of thick, tenacious pus, as well as cylindrical bronchial plugs, were aspirated. Through the inserted catheter, enzymes were instilled which led to liquefication of the thick bronchial secretion. Although this child had clinical evidence of improvement, he died about six weeks after the fenestration. Autopsy confirmed the clinical impression that he had hardly any functioning lung.

Obviously the aid provided by tracheal fenestration came too late. *This patient had temporarily benefited from tracheobronchial aspirations and demonstrated that liquefication of the thick mucus by endotracheal enzyme therapy is feasible.*

Case 3. J. H. was a 32-year-old Negro male with arrested far advanced pulmonary tuberculosis, bronchiectasis, and advanced emphysema (figure 5). His pulmonary tuberculosis was arrested by previous cavernostomy. His cough was excessive and he expectorated up to 500 ml. of purulent material daily. His dyspnea was such that he required continuous oxygen inhalation and he was completely bedridden.

On February 25, 1957, the patient was submitted to tracheal fenestration and a temporary tracheotomy. Aspirations with the "selective bronchial suction catheter" (3) (at times done by the patient) were performed at ten- to fifteen-minute intervals. His cough and the amount of tracheobronchial secretion diminished, and the patient was able to get out of bed and wheel himself around the corridors.

He had definite palliative benefit from the fenestration for the remaining three months of his life. *This patient benefited a great deal from the repeated tracheobronchial aspirations.*

Discussion

Tracheal fenestration with effective tracheobronchial aspirations and medication is a new hope for the respiratory cripple. With the help of repeatedly inserted special suction catheters (3), the evacuation of the tracheobronchial tree is more thorough than it would be by expectoration and, of course, it is no effort for the patient. The beneficial palliative effect of tracheal fenestration on the 3 desperate and moribund patients presented here in detail justified the additional clinical application of this technique (4).

The analysis of the 3 cases presented here is in order not only because it shows the clinical evolution of tracheal fenestration, but because it also serves as a guide in the present and future application of this procedure. It is obvious that the 3 patients of the study did not have enough unaltered lung left to give even the hope of lengthening their lives appreciably. On the other hand, it seemed possible that they could be made more comfortable by the repeated tracheobronchial aspirations and medications.

The experiences of the present writers with the first patient encouraged them not to abandon the far advanced case of tuberculosis with bilateral cavitary disease which is resistant to all of the available antituberculous drugs and is not accepted for surgical intervention because of the patient's low respiratory capacity. If such a patient is not found eligible even for cavernostomy (5) because of the numerous cavities present in both lungs, consideration of tracheal fenestration is in order. The patient should have roentgenographic evidence of enough

apparently unaltered lung to be able to live at least a sedentary life, providing the active disease is controlled. This, of course, was not the situation in the tuberculous patient presented here.

Cystic fibrosis of the pancreas with extensive pulmonary suppuration (6) in its terminal stage was the problem presented by the second moribund case. This 5½-year-old boy died of respiratory failure, as do others with this condition who survive the intestinal complications in infancy. The palliation provided by tracheal fenestration and repeated tracheobronchial aspirations clearly demonstrated that the thick, tenacious mucus can be removed not only from the central, but also from the peripheral, parts of the airways. The progressive decrease of the respiratory surface by the occluding endobronchial mucus can be slowed down, and it is believed that it can even be stopped. Of course, the earlier these children are exposed to the advantages of tracheal fenestration, the more chance they have for ultimate survival.

The third case represented a condition (advanced emphysema with marked pulmonary suppuration) in a stage when again only palliation could be accomplished. The decided benefit this patient gained from tracheal fenestration and repeated tracheobronchial aspirations demonstrated the logic of applying this method of treatment to patients in a somewhat less advanced state of their disease. To date, the present writers have employed tracheal fenestration in 12 additional patients. Five of these 12 patients had so-called advanced "dry" emphysema, and 7 had advanced emphysema with chronic suppurative bronchitis. The results of the treatment have been striking (7).

In contrast to tracheotomy, tracheal fenestration maintains a normal airtract. Accordingly, it does not interfere with phonation or with the cough mechanism. The surgically created tracheocutaneous fistula is closed at all times except when the skin valves are held apart. It is skin lined; therefore there is no need to keep its surfaces apart by a cannula in order to prevent re-growth or obliteration.

The experimental utilization of tracheal fenestration provides an opportunity for the exploration of the tracheobronchial tree. It can be utilized for the study of drug effects on the bronchial mucosa or for the production of various diseases, including suppuration and neoplasm. The easy insertion of a catheter or bronchoscope through the fenestration makes repeated endobronchial medication and observation possible. The effect of various test materials on the bronchial mucosa can be studied on animals with tracheal fenestration (8).

The indications for tracheal fenestration are: advanced cases of emphysema with or without suppuration; excessive suppurative diseases of the lungs, when excisional surgery is contraindicated; far advanced cases of pulmonary tuberculosis, for which no other form of therapy can be offered; fibrocystic disease of the pancreas with excessive pulmonary suppuration; and the bulbar type of poliomyelitis with irreversible changes requiring a mechanical respirator.

Summary

The rationale and the clinical and experimental application of tracheal fenestration have been presented. Experiences with this procedure were reported in 3

cases of advanced pulmonary insufficiency. One of the patients had far advanced pulmonary tuberculosis, one had cystic fibrosis of the pancreas with excessive pulmonary suppuration, and the third patient had advanced emphysema with widespread pulmonary suppuration. The observations indicate that tracheal fenestration with repeated tracheobronchial aspirations and medication offers definite therapeutic advantages.

Sumario

La Fenestración Traqueal como Nuevo Método para la Asistencia Terapéutica de las Afecciones Pulmonares Crónicas y para la Exploración Experimental del Arbol Bronquial

Preséntanse la evolución y la aplicación clínica y experimental de la fenestración traqueal. Descríbese lo observado con este procedimiento en 3 casos de insuficiencia pulmonar avanzada. Uno de los enfermos tenía tuberculosis pulmonar muy avanzada, otro tenía fibrosis quística del páncreas con excesiva supuración pulmonar y el tercero tenía supuración pulmonar diseminada. Las observaciones realizadas indican que la fenestración traqueal con repetidas aspiraciones traqueobronquiales y medicación ofrece ventajas terapéuticas bien definidas.

Resume

La fenestration trachéale, nouvelle méthode appliquée au traitement des maladies pulmonaires chroniques et à l'exploration expérimentale de l'arbre bronchique.

L'évolution, l'application clinique et expérimentale de la fenestration trachéale sont présentées; les auteurs rapportent l'expérimentation du procédé dans 3 cas d'insuffisance pulmonaire avancée. Un des patients présentait une tuberculose pulmonaire avancée, un autre une pancréatite fibro-kystique avancée et le troisième un emphysème pulmonaire avancé avec suppuration pulmonaire très étendue. Les observations indiquent que la fenestration trachéale avec aspirations trachéo-bronchiques répétées et médication offre des avantages thérapeutiques définis.

REFERENCES

(1) Rockey, E. E., Mayer, E., and Rappaport, I.: Tracheal fenestration, Dis. Chest, 1956, *30*, 226.

(2) Rockey, E. E.: Surgical technic of tracheal fenestration, Am. J. Surg., 1957, *94*, 486.

(3) Rockey, E. E., and Thompson, S. A.: Selective bronchial suction catheters and their clinical application, Am. J. Surg., 1958, *96*, 552.

(4) Rockey, E. E., Thompson, S. A., Blazsik, C. F., and Ahn, K. J.: Tracheal fenestration as a new method of treatment for advanced emphysema, New York State J. Med. (in press).

(5) Rockey, E. E., Thompson, S. A., and Shiner, I.: Cavernostomy, Am. Rev. Tuberc., 1948, *58*, 190.

(6) di Sant' Agnese, P. A.: Fibrocystic disease of the pancreas: A generalized disease of exocrine glands, J. A. M. A., 1956, *160*, 846.

(7) Rockey, E. E., and Thompson, S. A.: Present surgical technic of tracheal fenestration with a report of seventeen cases (in press).

(8) Rockey, E. E., Kuschner, M., Kosak, A. I., and Mayer, E.: The effect of tobacco tar on the bronchial mucosa of dogs, Cancer, 1958, *11*, 466.

PNEUMONECTOMY IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1]

T. W. SHIELDS,[2] W. M. LEES, AND R. T. FOX

(Received for publication June 3, 1958)

INTRODUCTION

The surgical resection of residual or persistent disease following adequate medical treatment has achieved a firm place in management of the individual with pulmonary tuberculosis. Excellent early as well as late results have been obtained following subsegmental, segmental, and lobar resections. However, the morbidity and mortality of pneumonectomy have remained high. The long-term follow-up studies of patients surviving pneumonectomy have revealed varying results.

MATERIAL

An analysis is presented of 101 patients who underwent pneumonectomy as a part of the treatment for pulmonary tuberculosis at the Chicago Municipal Tuberculosis Sanitarium during the years 1951 through 1955. The patients in whom pneumonectomy was performed for the treatment of carcinoma of the lung or for some other nontuberculous lesion were excluded from the study. One hundred and one patients were found suitable for inclusion in the study group. In addition to the hospital records, two- to five-year follow-up data were obtained on all patients surviving one year postoperatively.

A standard posterolateral thoracotomy with dissection and individual ligation of the hilar structures was employed. The bronchial stump was routinely closed with a single layer of interrupted fine silk sutures and was covered with an adjacent flap of pleura or mediastinal tissue. Drainage of the pleural space was not employed although the intrapleural pressure was checked and adjusted daily as necessary in the early postoperative period (usually for seven to ten days). A pleuropneumonectomy was performed when indicated. When bronchial secretions were a major problem, the operation was carried out in the prone position.

RESULTS

Sixty-six of the 101 patients undergoing pneumonectomy were women and 35 were men. At the time of operation, the youngest patient was thirteen years old and the oldest, seventy-two years of age. The majority of the patients were in the third and fourth decades. Forty-four of the patients were of European descent, 56 were Negroes, and one was of Asian descent. The known duration of the disease before pneumonectomy varied from one to nineteen years, the majority of the patients having had the disease from one to five years.

The indication for pneumonectomy was a destroyed lung in 34 patients, extensive disease throughout the lung in 32, thoracoplasty failure in 26, bronchiectasis with active tuberculosis in 2, extensive cystic disease subsequent to moderately advanced tuberculosis in one, massive pulmonary hemorrhage in one, and technical reasons during a contemplated lesser resection in 5 patients. Concomitant pleural or bronchial disease was present in a number of patients.

[1] From the City of Chicago Municipal Tuberculosis Sanitarium, Chicago, Illinois.
[2] Present address: Veterans Administration Research Hospital, Chicago, Illinois.

The pneumonectomy was performed on the right in 43 patients and on the left in 58 patients. The sputum was positive for acid-fast organisms at the time of surgery in 45 (44.5 per cent) and negative in 56 (55.5 per cent) of the patients. Acute endobronchitis had been diagnosed in 52 patients at some time during the disease. As a rule, this had subsided on appropriate treatment before resection was performed. In some, however, the ideal presence of a healed bronchus was not obtained either on the most recent bronchoscopic examination prior to operation or on histologic examination of the resected stump.

During the early period of study, antimicrobial drugs were used in various combinations and for various lengths of time. Streptomycin alone or with PAS was used most often; isoniazid was added to most regimens when it became available. On occasion, viomycin and pyrazinamide were employed in the immediate preoperative period and for one to two months postoperatively. Sixty-three patients received continuous antimicrobial therapy, whereas 38 had interrupted treatment.

Eleven patients died within sixty days of the surgical procedure, a postoperative mortality of 10.8 per cent. An additional 5 patients (4.9 per cent) died in the hospital within one year as a result of either the complications or sequelae of the operation. Eighteen patients (17.8 per cent) developed one or more major nonfatal complications; in only 2 patients were these nontuberculous in nature. Ten patients (9.9 per cent) sustained minor complications and 57 patients (56.4 per cent) had uneventful postoperative courses.

Of the 85 patients who survived, 37 were discharged with inactive disease, 20 with arrested disease, 11 with active disease improved with a good prognosis, 9 with active disease, but having reached maximal hospital benefit; 6 left the Sanitarium against medical advice, and 2 patients died after a prolonged hospital stay of more than twenty-four months.

Hospital deaths: A total of 16 patients died in the hospital within one year of the pneumonectomy. A right pneumonectomy was performed in 10 and a left pneumonectomy in 6 patients.

Of the 11 patients who were dead within sixty days of the pneumonectomy, 6 died as the result of cardiorespiratory failure. This was manifested by increasing dyspnea, cyanosis, and terminally by acute pulmonary edema. In 2 of these 6 patients, reactivation or spread of contralateral disease played a major role in the development of respiratory insufficiency. One patient had a known mitral valve lesion (combined stenosis with regurgitation), but had received medical clearance for the surgical procedure. Pulmonary function studies had been done in 3 of the 6 patients. In each, the pulmonary reserves were diminished but were believed to be adequate. The duration of survival of these 6 patients following pneumonectomy was from six hours to twenty-four days.

The 5 remaining patients of this group died as a result of the following causes: uncontrollable hemorrhage at surgery, secondary hemorrhage from the pulmonary arterial stump, persistent shock, anuria with terminal pulmonary edema, and a bronchopleural fistula with empyema.

Five patients died more than sixty days after operation, but death occurred

TABLE 1

INCIDENCE OF SPUTUM POSITIVE FOR TUBERCLE BACILLI, RE-TREATMENT WITH ANTIMICROBIALS, AND PREVIOUS ENDOBRONCHITIS IN THE PATIENTS UNDERGOING PNEUMONECTOMY

	Number of Patients	Sputum Positive for Tubercle Bacilli	Re-Treatment with Antimicrobials	Previous Endobronchitis
Hospital deaths	16	7	7	12
Major morbidity	18	12	8	10
Minor morbidity	10	3	3	3
Uncomplicated	57	23	20	27
Total	101	45	38	52

TABLE 2

DISCHARGE STATUS OF PATIENTS UNDERGOING PNEUMONECTOMY FOR PULMONARY TUBERCULOSIS

	Number of Patients	Disease Inactive	Disease Arrested	Disease			
				Active: Good Prognosis	Active MHB*	Active AMA†	Deaths
Hospital deaths	16	0	0	0	0	0	16
Major morbidity	18	5	3	0	7	1	2
Minor morbidity	10	4	1	3	1	1	0
Uncomplicated	57	28	16	8	1	4	0
Total	101	37	20	11	9	6	18

* Active MHB: Active disease with maximal hospital benefit

† AMA: Irregular discharge, against medical advice

in the hospital within the first year following the pneumonectomy. Contralateral reactivation or spread of the disease was the predominant complication in 4 of these patients; empyema with a bronchopleural fistula was also present in 2 of these 4 patients. The remaining patient died of progressive deterioration resulting from a bronchopleural fistula and empyema, for which the patient refused additional surgical therapy other than drainage.

Major morbidity: The postoperative course in 18 patients was abnormal in that one or more major, nonfatal complications occurred in each. In 16 patients the complications were tuberculous. Reactivation or spread of contralateral disease occurred ten times, a bronchopleural fistula with empyema five times, and empyema without bronchial breakdown twice. Nontuberculous, major complications occurred in 2 patients. In one patient cardiac decompensation occurred, and in the other patient serum hepatitis with jaundice developed.

The status of the sputum, the adequacy of antimicrobial therapy, and the presence of previous acute endobronchitis are tabulated in table 1. As previously noted, 2 of these patients died in the hospital twenty-four months after pneumonectomy. The activity of the tuberculosis of the 16 living patients at

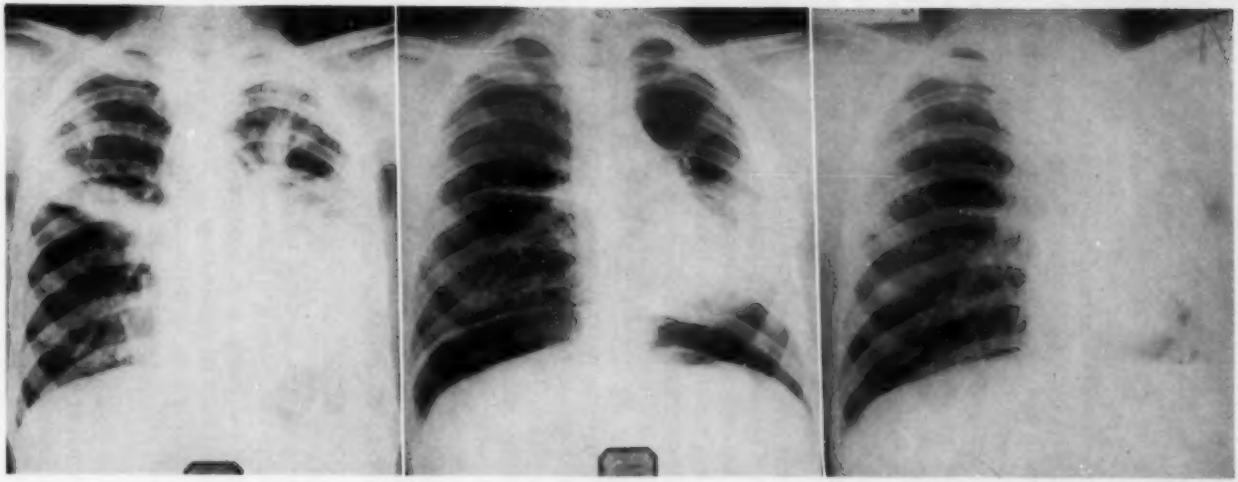


FIG. 1. L. F., 26-year-old Negro male. (Left) Admission film; sputum positive for tubercle bacilli. (Center) Preoperative film after chemotherapy for thirteen months and pneumoperitoneum. (Right) Film obtained three and a half years postoperatively. Patient's disease is inactive; he is working full time.

the time of discharge is tabulated in table 2. The follow-up observation of these patients reveals that the disease is inactive in 9, active in 2 patients who are working, and that 5 patients are dead. The cause of death in each was directly related to the patient's disease or the complications of the surgical procedure.

Minor morbidity: Ten patients sustained minor complications which were all nontuberculous in character. These complications were: a temporary brachial plexus injury, postoperative bleeding or persistent hypotension readily corrected by transfusions, excessive and somewhat prolonged tachycardia or fever, and temporary wound drainage. The studies preoperatively and the status at discharge from the hospital are shown in tables 1 and 2, respectively. Nine patients have been followed for two or more years. All are alive and have inactive disease. The remaining patient whose disease was also inactive died of a ruptured appendix at another hospital twenty-three months following the pneumonectomy.

Uncomplicated postoperative course: Fifty-seven patients were considered to have experienced essentially normal postoperative courses following pneumonectomy. The preoperative studies and discharge status are recorded in tables 1 and 2. Seven of the discharged patients could not be traced. Of the 50 patients followed, 48 are now classed as having inactive disease, one has active disease, and the remaining patient died of uremia at another hospital fifty-four months after the pneumonectomy.

Surgical Follow-up Data

Ninety patients survived the pneumonectomy for a period longer than sixty days. Five patients died within one year, and 2 patients died more than twenty-four months after operation. These 7 patients never left the Sanitarium postoperatively. Eighty-three patients left the hospital alive. Seven of these 83 patients are known to have died; 5 of their tuberculous disease and 2, of unrelated causes. Seven additional patients could not be traced.

The 2- to 7-year follow-up data on the 69 known living patients revealed the disease to be inactive in 65 and active in 4. Dyspnea is reported to be significant in only 2 patients of the surviving group.

Tuberculous Complications

An analysis of the fatal and nonfatal tuberculous complications revealed that reactivation or spread of contralateral disease occurred sixteen times (15.8 per cent), a bronchopleural fistula with empyema nine times (8.9 per cent), and empyema without bronchial fistula twice (1.9 per cent). These twenty-seven complications occurred in 24 patients. Reactivation of contralateral disease was associated with a bronchopleural fistula in 2 patients and with an empyema in one.

Tubercle bacilli were present preoperatively in the sputum of 13 of the 16 patients with reactivation or spread of contralateral disease. The antimicrobial therapy had been interrupted and re-treatment with drugs for one or more periods had been carried out in 9 patients. In addition, acute endobronchitis

TABLE 3

INCIDENCE OF SPUTUM POSITIVE FOR TUBERCLE BACILLI, RE-TREATMENT WITH ANTIMICROBIALS, AND ACUTE ENDOBRONCHITIS AT THE TIME OF PNEUMONECTOMY IN PATIENTS WHO DEVELOP TUBERCULOUS COMPLICATIONS AND IN THOSE WITH NO TUBERCULOUS COMPLICATIONS

	Number of Patients	Sputum Positive for Tubercle Bacilli	Re-Treatment with Antimicrobials	Acute Endobronchitis
		per cent	*per cent*	*per cent*
Contralateral spread	16	81.2	56.2	37.5
Bronchopleural fistula	9	55.5	44.4	71.1
Empyema	2	100.0	0	
No complications*	69	37.6	33.3	7.2

* Exclusive of early postoperative deaths

was present in 6 patients at the time of the pneumonectomy. On reviewing the preoperative chest roentgenograms, it was noted that increasing contralateral disease in 2 patients had been improperly evaluated prior to resection. Four of the 10 patients originally surviving one year following this complication have died of progressive disease and its subsequent sequelae. The tuberculous process in the 6 living patients of this group is now classified as inactive in 5 and active in one.

Nine patients developed bronchopleural fistulas. The manner of bronchial closure did not significantly vary from that utilized in the other patients in the study. Seven of the 9 patients were discovered to have had acute endobronchitis at the time of the most recent bronchoscopy prior to surgery or on histologic examination of the bronchial stump. In addition, the original course of chemotherapy had been interrupted in 4 patients, and the preoperative sputum of 5 of the 9 patients was found to contain tubercle bacilli. The follow-up data on the 5 patients of this group discharged from the hospital revealed that 3 additional patients had died of their disease.

Empyema occurred in 2 patients, one in association with reactivation of contralateral disease. Both patients had previous thoracoplasties and in each the sputum was positive for tubercle bacilli preoperatively. The original antimicrobial therapy had not been interrupted nor had endobronchitis been discovered in either patient. Both patients are alive, although one continues to have drainage from the empyema space intermittently positive for acid-fast bacilli.

A comparison of the sputum status, continuity of the chemotherapy, and the presence of acute endobronchitis in the patients with tuberculous complications and all other patients surviving the early postoperative period are recorded in table 3.

DISCUSSION

In the present series of 101 pneumonectomies performed in the treatment of pulmonary tuberculosis, it remains evident that the procedure is a formidable one. The extensive pathologic involvement necessitating the procedure

and the apparent lack of sufficient natural resistance of the patient to his disease make the situation hazardous. Moreover, the frequent involvement of the contralateral lung by previous disease with concomitant decrease in pulmonary reserve makes the necessary physiologic adjustments of the cardiorespiratory mechanics difficult. Yet, in such a group of patients, in whom both medical and lesser surgical procedures have failed to control the disease process, such a high-risk procedure is justified in an attempt to salvage the patient so that he may return to his place in society.

Cardiorespiratory failure was the most common cause of early postoperative death. With application of routine pulmonary function studies, however, most deaths of this type may be eliminated. In the occasional patient in whom such an event occurs or is precipitated by extension of contralateral disease, the employment of a mechanical respirator as advocated by Bjork and Engstrom (1) may be life saving. Recently, it has been possible to foretell such a complication by the use of cardiac catheterization with a balloon type of catheter.

Tuberculous complications, i.e., contralateral disease, bronchopleural fistula, and empyema, continue to occur frequently. However, in no case was ulceration of the bronchial stump noted. The discrepancy between the present findings and those of Robinson and Corpe (2) and Schlosser and Jarvis (3) in this respect is unexplained.

The high incidence of infectious sputum and also the high incidence of retreatment with antimicrobials in those patients with tuberculous complications as contrasted with those having no complications are readily apparent from table 3. Unfortunately, drug-resistance studies were not carried out in most patients of this group. However, in patients with sputum positive for tubercle bacilli following interrupted and repeated antimicrobial therapy, it may be assumed that the incidence of resistance is high. Undoubtedly, this played a major role in those patients who developed postoperative complications and is in agreement with the study of Steele (4) as to the adverse effect of drug-resistant organisms in the postoperative course.

The complete avoidance of exacerbation of contralateral (occasionally spread) disease is difficult to achieve in such a group of patients. It should be suspected as a possibility, however, in those with resistant organisms and in those who have had previous extensive disease in the contralateral lung. Preoperative treatment with a different chemotherapeutic regimen is indicated in this group of patients. Also, clinical and roentgenographic stability (not obtained in 2 of the present group) must unquestionably be present before the operation is performed. The "spill-over" of infected secretions must also be avoided; this is best achieved by the use of the prone position for the patient during operation, and early division and closure of the bronchus.

Bronchopleural fistula and empyema are related to the presence of both infectious sputum and acute endobronchial disease. Previous but healed endobronchitis was more frequent in the patients who died or sustained major tuberculous complications than in the patients without complications. However, this manifestation probably is related more to the severity of the original disease process than to the outcome of the surgical procedure per se.

No bronchial breakdown occurred as a result of faulty closure. All were the result of a primary failure of healing or were secondary to an empyema that eroded into the bronchial stump. The presence of acute endobronchitis in many of the cases is believed to be a definite factor in the failure of a bronchus to heal. The present writers agree with Hardy and Sampson (5) that the bronchus should be in a quiescent state before resection is attempted. If this condition is not obtained by appropriate therapy and the endobronchitis remains acute, as interpreted by bronchoscopic visualization, then resection should be deferred. In those patients in whom the bronchial disease appears inactive on bronchoscopy prior to operation but is found to be active at surgery, steps should be taken to forestall possible breakdown of the stump. During the operation, additional protection for the stump may be obtained by transplantation of a pleural flap or the pericardial fat pad. The latter has been shown to be of definite value by the studies of Brewer and Bai (6). If bronchial disease is not discovered until the postoperative period, i.e., histologic examination of the bronchus, then an elective thoracoplasty to obliterate the pleural space would seem to be of value. This is considered in the early postoperative period if the patient exhibits signs of impending infection: fever, cough, rapid accumulation of fluid in the hemithorax, or a change in the character of this fluid. It should be mentioned that the use of routine postoperative thoracoplasty is not indicated and has not been utilized by the present writers. The studies of Hirdes and Bosch (7) support this contention.

Major nontuberculous complications, if successfully treated, and minor postoperative complications had no adverse effect on the long-term benefit of the pneumonectomy. These patients, as well as those in whom the postoperative course was uneventful, had excellent prognoses for successful, long-term survival. The high incidence of sputum negative for tubercle bacilli, the presence of an original treatment course, and the infrequent occurrence of acute endobronchitis, as shown in table 3, appear to be significant in this group.

In view of the above information, it is obvious that strict adherence to the basic principles of surgery in pulmonary tuberculosis is still paramount. These principles, as laid down by the pioneers in this field, have been modified to some extent by antimicrobials and improved anesthetic and surgical techniques, but are still quite valid in their basic rationale. At present, all of the patients for whom pneumonectomy is proposed are evaluated carefully by pulmonary function studies, bronchoscopy, electrocardiograms, and routine blood studies. If the sputum is infectious, the antimicrobial regimen is changed to include at least one and preferably two agents which have not previously been used on this patient. A "quiescent" bronchus and roentgenographic stability of disease are mandatory before resection is undertaken.

Summary

The records of 101 patients subjected to pneumonectomy in the treatment of pulmonary tuberculosis have been reviewed and 2- to 7-year follow-up data of survivors have been obtained. The sixty-day mortality was 10.8 per cent. Fourteen of the 90 patients surviving the early postoperative period are known to be

dead and 7 were lost to follow-up observation after discharge from the Sanitarium. Sixty-nine patients are known to be alive, 65 with inactive disease; the activity of the tuberculous process persists in 4 patients.

Cardiorespiratory failure was the most common cause of early death. Tuberculous complications were frequent causes of late mortality; their occurrence indicates a poor prognosis. The incidence of infectious sputum, interrupted antimicrobial therapy, and acute endobronchitis was high in this group. This is in contrast to the lower incidence of these findings in the patients who sustained either no complications or minor nontuberculous complications, and in the surviving patients it is of good prognostic significance. In the latter patients, satisfactory long-term survival with inactive disease is the usual course.

Sumario

La Neumonectomía en el Tratamiento de la Tuberculosis Pulmonar

Se han repasado los protocolos de 101 enfermos sometidos a la neumonectomía para el tratamiento de la tuberculosis pulmonar, acompañado esto de una observación de dos a siete años en los sobrevivientes. La mortalidad de sesenta días fué de 10.8 por ciento. Se sabe que 14 de los 90 que sobrevivieron el período postoperatorio temprano han fallecido y 7 fueron perdidos de vista después del alta del Sanatorio. Se sabe que 69 están vivos, 65 de ellos con enfermedad inactiva; la actividad del proceso tuberculoso persiste en 4 sujetos.

La insuficiencia cardiorrespiratoria fué la causa más común de la muerte temprana. Las complicaciones tuberculosas fueron causas frecuentes de mortalidad tardía; su aparición indica un pronóstico malo. La incidencia de esputo infeccioso, terapéutica antimicrobiana interrumpida y endobronquitis aguda fué elevada en este grupo. Esto es en contraposición a la incidencia más baja de estos hallazgos en los enfermos que bien no tuvieron complicaciones o sólo tuvieron pequeñas complicaciones no tuberculosas, lo cual tiene en los sobrevivientes buen significado pronóstico. En los últimos enfermos, lo habitual es una sobrevivencia satisfactoria a largo plazo con enfermedad inactiva.

Resume

Pneumectomie dans le traitement de la tuberculose pulmonaire

Les dossiers de 101 malades soumis à la pneumectomie pour traitement d'une tuberculose du poumon ont été examinés; on a ainsi obtenu des observations sur les survivants qui ont été suivis pendant des périodes de deux à sept ans. Le taux de mortalité en soixante jours était 10, 8%. Parmi les 90 malades survivant au début de la période préopératoire, 14 sont morts et 7 ont été perdus de vue après leur départ du sanatorium. Soixante-neuf patients sont vivants, 65 présentent une tuberculose stabilisée, le processus évolutif persiste chez 4 patients.

La défaillance cardio-respiratoire était la cause la plus fréquente de la mort précoce. Les complications tuberculeuses étaient les causes les plus communes de la mortalité tardive, leur survenue est d'un mauvais pronostic. L'incidence de crachats bacillifères, de l'interruption de l'antibiothérapie et de l'endobronchite aiguë était très élevée dans ce groupe. Ceci contraste avec l'incidence plus réduite de ces éléments chez les malades indemnes de complications, ou présentant des complications mineures non tuberculeuses, et le pronostic est bon chez les malades qui ont survécu. Chez ces derniers, on observe habituellement la survie prolongée avec tuberculose stabilisée.

REFERENCES

(1) Bjork, V. O., and Engstrom, C. G.: The treatment of ventilatory insufficiency after pulmonary resection with tracheostomy and prolonged artificial ventilation, J. Thoracic Surg., 1955, *30*, 356.

(2) Robinson, J. S., and Corpe, R. F.: Pneumonectomy in pulmonary tuberculosis, Am. Rev. Tuberc., 1958, *77*, 73.

(3) Schlosser, R. J., and Jarvis, F. J.: Resection failures in pulmonary tuberculosis, J. Thoracic Surg., 1955, *29*, 335.

(4) Steele, J. D.: U. S. Veterans Administration-Armed Forces Cooperative Studies of Tuberculosis: IV. Results of pulmonary resection, Am. Rev. Tuberc., 1956, *73*, 960.

(5) Hardy, K. L., and Samson, P. C.: "Quiescent" tuberculous bronchus: Review with study of its surgical connotations, Am. Rev. Tuberc., 1956, *73*, 451.

(6) Brewer, L. A., and Bai, A. F.: Surgery of the bronchi and trachea: Experience with the pedicled pericardial fat graft reinforcement, Am. J. Surg., 1955, *89*, 331.

(7) Hirdes, J. J., and Bosch, M. W.: Pneumonectomy in pulmonary tuberculosis without thoracoplasty, J. Thoracic Surg., 1955, *30*, 719.

SEVEN- TO ELEVEN-YEAR FOLLOW-UP STUDY OF THE RESULTS OF PRIMARY THORACOPLASTY FOR PULMONARY TUBERCULOSIS[1]

The Relationship of Certain Variables of the Disease to Outcome

HORACE H. ZINNEMAN AND WILLIAM B. TUCKER[2]

(Received for publication July 22, 1958)

INTRODUCTION

Surgical collapse therapy has been a prevalent method in the treatment of parenchymal pulmonary tuberculosis. The paucity of reports of late results is remarkable, however, and most follow-up reports are restricted to the well-being, disability, or death of patients at the time of study. Kinsella (1), Adie (2), Gutheil (3), and Refsum (4) and their associates are among the writers whose studies represent late follow-up results.

It seemed worth while to review the late follow-up results of a group of patients who underwent primary thoracoplasty for pulmonary tuberculosis at Minneapolis Veterans Administration Hospital during the period from 1946 to 1950. By the nature of the institution and its follow-up methods, these patients form a well-controlled group which permitted a study, not only in respect to the general status of health but also continued close surveillance by roentgenographic, bacteriologic, and physical examinations. Above all, the homogeneity of this group encouraged an attempt at analysis of the influence of certain variables of the disease upon the late outcome.

MATERIALS AND METHODS

All patients received streptomycin and PAS for a period of one to three months during the pre- and postoperative phases. None of the patients had long-term chemotherapy as it is known now. The severity and extent of the pulmonary involvement at the time of surgery were classified according to the *Diagnostic Standards* of the National Tuberculosis Association (1955 edition). A primary seven-rib, three-stage thoracoplasty was the chief surgical procedure employed for the control of pulmonary tuberculosis during the period from 1947 to 1950. The entire first rib and the long posterolateral segments of the second, third, and fourth ribs were removed subperiosteally during the first stage of the operation. The ribs were resected well within the transverse vertebral processes. During the second stage, the anterior parts of the second, third, and fourth ribs were resected, with excision of the costal cartilages up to the sternum. The posterolateral segments of the fifth, sixth, and seventh ribs were removed subperiosteally during the third stage. The three surgical stages were performed at two-week intervals.[3] In 1947, three lobectomies and one penumonectomy were performed; this increased to a total of 49 for the four-year span ending in 1950. A marked increase in the amount of excisional surgery began in 1951, and only a rare primary thoracoplasty was performed during and after 1951.

---

[1] From the Veterans Administration Hospital, Minneapolis, Minnesota; and the Department of Medicine, University of Minnesota, Minneapolis, Minnesota.

[2] Present address: Director, Tuberculosis Service, Veterans Administration, Washington 25, D. C.

[3] The surgery was performed under the guidance and cooperation of T. J. Kinsella and N. K. Jensen.

TABLE 1

FIFTY-EIGHT PATIENTS ELIMINATED FROM STUDY

Total number of cases		200
Excluded from study because		
Thoracoplasty was adjunct to resection	21	
Thoracoplasty performed elsewhere	4	
Thoracoplasty for correction of empyema	4	
Thoracoplasty for correction of unexpanded lung	4	
Other variances	2	
Incomplete records or films	23	
		58
Cases remaining for analysis		142

During the period of hospitalization a minimum of six cultures (5) from sputum, three from gastric washings, and one chest roentgenogram were examined each month. Follow-up examinations after discharge included chest roentgenograms (often planigrams), cultures of the sputum or gastric washings, urinalyses, hemograms, as well as physical examinations at regular intervals. The term "conversion" as used in this study signifies a reversal of infectiousness by culture, maintained throughout the follow-up period. "Failure" signifies either the failure to "convert" by culture initially or "reconversion" to cultures positive for tubercle bacilli later on. When a subsequent procedure was added, such as a resection, the bacteriologic result at the time of resection was tabulated.

Fifty-eight patients were eliminated from this study in order to restrict it to parenchymal pulmonary lesions, treated and followed under comparable circumstances (table 1). The remaining 142 cases represent a uniform group. The period of follow-up study ranged from seven to eleven years. Only 2 patients were lost to long-term observation. Both had attained noninfectious status at the time of their last examination and appear in this study under "conversions."

Thirteen deaths, none of them in the immediate postoperative period, are included in the group of "failures," since the deaths were a sequence of miliary spread or an extension of the disease, or the effect of the surgical procedure on the respiratory capacities of the patients.

RESULTS

In table 2 it may be seen that only 6 patients were classified as having minimal disease. Primary thoracoplasty resulted here in 5 "conversions." Eighty-four patients had moderately advanced, and 52 far advanced, disease. Only 44 per cent of all patients "converted" by the criteria listed in "Materials and Methods." The differences between unilateral and bilateral disease are not striking. As may be expected, the percentage of "conversion" for far advanced cases is materially smaller than that for the moderately advanced group. As mentioned previously, the 79 "failures" include 13 deaths. Thirty-three of the "failures" subsequently underwent resective surgery, which was performed on the side of the original thoracoplasty. Most of the remaining 33 "failures" have since been placed on long-term chemotherapy.

Table 3 represents a correlation of the total diameter of cavities with the total diameter of pulmonary nodules seen on roentgenograms with regard to their bearing and influence on "conversion" after primary thoracoplasty. It

TABLE 2

Late Results of Primary Thoracoplasty for Pulmonary Tuberculosis

Stage of Disease	Number of Patients	Number of Lungs Involved		Late Result Bacteriologically "Conversion"*		"Failure"*	
				Number	Per Cent	Number	Per Cent
Minimal	6	Unilateral	6	5	†	1	†
		Bilateral	0	0		0	
		Subtotal	6	5	†	1	†
Moderately advanced	84	Unilateral	55	26	47	29	53
		Bilateral	29	18	62	11	38
		Subtotal	84	44	52	40	48
Far advanced	52	Unilateral	15	5	33	10	67
		Bilateral	37	9	24	28	76
		Subtotal	52	14	27	38	73
All stages	142	Unilateral	76	36	47	40	53
		Bilateral	66	27	41	39	59
Total			142	63	44	79	56

* "Conversion" signifies reversal of infectiousness by culture, maintained throughout follow-up period; "failure" signifies either failure to "convert" by culture initially, or "reconversion" subsequently to positive. When subsequent procedure is added, such as resection, the bacteriologic result at the time of resection is tabulated.

† Percentages not calculated when the number of cases was less than 15.

Note: Of 79 "failures," 13 died and 33 subsequently underwent resection, in every instance for disease on the side of the thoracoplasty, with "conversion" bacteriologically in every case.

TABLE 3

"Sputum Conversion" After Primary Thoracoplasty for Pulmonary Tuberculosis

Correlation of Total Diameter of Cavities with Total Diameters of Nodules

Total Diameter of Cavities		Total Diameter of Nodules in cm.								Total		Per Cent "Converted"
		0		<1.0		1–2.9		3 and More				
		"Conversion"	Total	"Conversion"	Total	"Conversion"	Total	"Conversion"	Total	"Conversion"	Total	
cm.												
0	"Conversion"	0		0		5		8		13		72
	Total		0		0		7		11		18	
<1.0	"Conversion"	0		0		4		3		7		54
	Total		0		0		7		6		13	
1–2.9	"Conversion"	0		0		15		11		26		43
	Total		4		0		28		28		60	
3 and more	"Conversion"	0		0		2		15		17		33
	Total		4		0		15		32		51	
All	"Conversion"	0		0		26		37		63		
	Total		8		0		57		77		142	
Per cent "converted"		0		0		46		48				44

TABLE 4

"SPUTUM CONVERSION" AFTER PRIMARY THORACOPLASTY FOR PULMONARY TUBERCULOSIS

Correlation of Total Diameter of Cavities with Number of Lungs Involved

Total Diameter of Cavities	Lung(s) Involved				Total		Per Cent "Converted"
	Unilateral		Bilateral				
	"Conversion"	Total Number	"Conversion"	Total Number	"Conversion"	Total Number	
cm.							
0	9	12	4	6	13	18	72
<1.0	5	9	2	4	7	13	54
1-2.9	15	36	11	24	26	60	43
3 and more	7	19	10	32	17	51	33
Totals	36	76	27	66	63	142	
Per cent "converted"	47		41				44

Note: Analysis of relationship to total diameter of nodules, for unilateral and bilateral cases separately, as in table 2 for all cases, does not reveal a significant relationship.

TABLE 5

"SPUTUM CONVERSION" AFTER PRIMARY THORACOPLASTY FOR PULMONARY TUBERCULOSIS

Relationship Between "Acuteness" of Disease and Bacteriologic "Conversion" or "Failure"

Grades of "Acuteness"	Number of Cases	Number "Converted"	Number "Failure"	Per Cent "Converted"
1-3	24	10	14	42
4-6	82	38	44	46
7-9	36	15	21	42
Total	142	63	79	44

Note: Cases of unilateral and bilateral disease tabulated separately show approximately the same relationship as for all cases in the table above, without significant difference.

became obvious that the total diameter of nodules in patients with nodules of more than 1 cm. in diameter does not appear to influence the "conversion" rate, whereas there is an excellent correlation between the diameter of cavities and "conversion": the smaller the cavity the better the long-range result of primary thoracoplasty. Separate analyses of unilateral and bilateral pulmonary disease followed the same pattern. The composite results of these analyses are presented in table 4. No correlation was found in an additional analysis between the diameter of the largest nodules and "conversion."

A correlation of the acuteness of the pulmonary disease at the time of surgery with the end result after primary thoracoplasty is shown in table 5. The stages of acuteness were established in accordance with the *Diagnostic Standards*

of the National Tuberculosis Association (1955 edition), and were determined jointly by the present writers upon a careful review of all roentgenograms. During the earlier years of the period of study, patients with acute disease were not subjected to surgery. This caution was relaxed later on and justification for this step is furnished in table 5, since virtually no influence of the acuteness of the disease is shown on the final result.

Discussion

The high incidence of "failure" encountered in this study is at variance with most follow-up studies of primary thoracoplasty (1–3, 6–11). In figure 1 it may be seen that the "failure" rate in this study was 38 per cent after one year, and that it gradually rose to 56 per cent during the following years. The criteria for bacteriologic conversion were more rigid than those for other studies, some of which were based on the subjective well-being of the patient, chest roentgenograms, or microscopy of the sputum. The occasional finding of acid-fast bacteria on smears was viewed without concern in some of the follow-up studies (4, 10). Other studies covered only a short follow-up period (6, 7, 11); still others were restricted to the survival period of patients after primary thoracoplasty (9). The average "failure" rate of these follow-up studies is about 30 per cent. It is believed that the high "failure" rate of 56 per cent in the present series is merely the expression of strict criteria for "conversion."

The writers do not believe that an occasional sputum containing acid-fast bacteria on microscopy is to be viewed without alarm, particularly since two of the late deaths in this series were due to a miliary spread of the disease.

It was apparent that the more rigid criteria of this study contributed to the relatively high "failure" rate reported. Nevertheless, even disregarding the effect of the meticulous bacteriology, the failure of an appreciable number of patients to reverse their infectiousness was disappointing. This review afforded an opportunity to attempt a retrospective analysis to determine

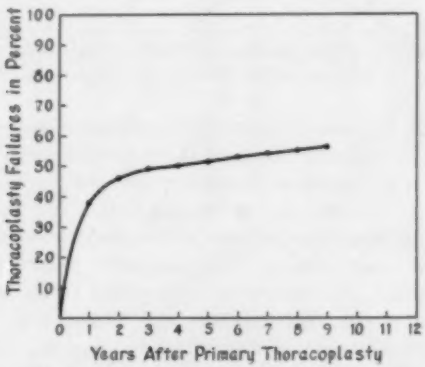


Fig. 1. The incidence of primary thoracoplasty failures in relation to the time interval since surgery. Most of the failures became evident within twenty-four months following thoracoplasty.

whether certain factors characterizing the disease might be related to the outcome.

This analysis has included three variables determined from careful serial analysis of roentgenograms: (*a*) the "acuteness" of the tuberculous disease, (*b*) the size of residual tuberculous nodules, and (*c*) the diameter of tuberculous cavities. It has been found that, within the range of the clinical material here presented, "acuteness" and diameter of nodules—either total diameter of all nodules or diameter of the largest nodule—appear not to be correlated with the "failure" rate. In contrast, there appears to be a definite correlation with the total diameter of the cavities. The influence of the variable as to whether tuberculous disease existed in one lung or in both was more difficult to evaluate. In all probability the relatively small difference in results between unilateral and bilateral cases can be explained by selective factors: In the majority of cases of bilateral disease, thoracoplasty was carried out on the more extensively involved side, with relatively little, and relatively stable, disease in the contralateral lung.

The bacteriologic "conversion" rate of 44 per cent in primary thoracoplasties compares unfavorably with the "conversion" rate of 95 per cent in a series of surgical resections performed by the same team (12). It is admitted that the chemotherapeutic regimen of the two groups was not the same, that of the resections being of longer duration and of greater variety. Still, the experiences with primary thoracoplasty for pulmonary tuberculosis persuaded the writers to abandon this procedure in 1951 in favor of pulmonary resection.

Summary

In a seven- to eleven-year follow-up study of 142 primary thoracoplasties for pulmonary tuberculosis, it was found that acuteness of the disease and the diameter of nodules appeared not to be correlated with "failure." In contrast, there appeared to be a definite correlation with the total diameter of cavities. The failure of 56 per cent of the patients to reverse their infectiousness was disappointing.

Sumario

Siete a Once Años de Observación de los Resultados de la Toracoplastia Primaria por Tuberculosis Pulmonar: La Relación de Ciertas Variables con el Desenlace

En un estudio de siete a once años de observación de 142 toracoplastias primarias por tuberculosis pulmonar, se descubrió que la agudeza de la enfermedad y el diámetro de los nódulos no parecen correlacionarse con el "fracaso." En contraposición, parece existir una correlación bien definida con el diámetro total de las cavernas. Fué desalentador el hecho de que, en 56 por ciento de los enfermos, no virara la infecciosidad.

Resume

Sept à onze ans d'observation des résultats de la thoracoplastie primaire pour tuberculose pulmonaire: Corrélation entre certaines variables de l'affection et son issue

Au cours d'une étude fondée sur l'observation periodique de 142 thoracoplasties primaires pour tuberculose pulmonaire, on a observé l'absence de corrélation entre la forme aiguë de l'affection, le diamètre des nodules et les "échecs." Par contre, il apparaît qu'il existe

une corrélation définie avec le diamètre total des cavernes. La suppression de l'infectiosité n'a pas été obtenue chez 56% des malades ce qui a causé une déception.

REFERENCES

(1) Kinsella, T. J., Mariette, E. S., Matill, P. M., Fenger, E. P. K., Funk, V. K., Larson, L. M., Cohen, S. S., and Nemce, F. C.: Thoracoplasty in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1949, *59*, 113.

(2) Adie, G. C., Childress, W. G., Brezing, H. J., and Taylor, D.: Late results in the treatment of pulmonary tuberculosis by thoracoplasty, J. Thoracic Surg., 1952, *23*, 92.

(3) Gutheil, D., Steele, J. D., Cadden, A. V., and Sakaguchi, S.: Late results of thoracoplasty according to type of pulmonary tuberculosis, Am. Rev. Tuberc., 1950, *62*, 645.

(4) Refsum, E.: Thoracoplasty in cavernous tuberculosis: Clinical statistics, study of late results, Acta tuberc. scandinav., 1951 (Supplement 29, p. 3).

(5) Diagnostic Standards and Classification of Tuberculosis, National Tuberculosis Association, New York, 1955, pp. 52–53.

(6) Rubin, M., and Klopstock, R.: Influence of type of disease on the results of thoracoplasty in pulmonary tuberculosis, Am. Rev. Tuberc., 1949, *60*, 273.

(7) Steele, J. D.: The early use of surgical procedures in the treatment of pulmonary tuberculosis, California Med., 1950, *73*, 551.

(8) Shaw, R. R., and Paulson, D. L.: Indications for thoracoplasty and resection in pulmonary tuberculosis, Nat. Tuberc. A. Tr., 1950, pp. 255.

(9) Brantigan, O. C.: Thoracoplasty in the treatment of pulmonary tuberculosis, Am. Surgeon, 1952, *18*, 8.

(10) Douglass, R., and Bosworth, E. B.: Thoracoplasty: Ten year follow-up, Nat. Tuberc. A. Tr., 1952, pp. 256.

(11) Hughes, F. A., and Polk, J. W.: Thoracoplasty and resection for pulmonary tuberculosis, J. Thoracic Surg., 1953, *25*, 454.

(12) Falk, A., and Jensen, N. K.: Bilateral resection in pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *74*, 367.

AN ANALYSIS OF PREOPERATIVE BRONCHIAL BIOPSY IN ONE HUNDRED PATIENTS WITH PULMONARY TUBERCULOSIS[1,2]

NEIL C. ANDREWS, CLARENCE I. BRITT, AND PHILIP C. PRATT

(Received for publication May 29, 1958)

INTRODUCTION

In 1956, Andrews and associates (1) reported an analysis of 100 patients with pulmonary tuberculosis in which the postresectional complications of bronchial origin were correlated with the submucosal findings in the resected bronchus at the point of transection. These bronchi showed either normal tissue, lymphocytic infiltration, or tubercle formation within the submucosa. It was observed that no complication of bronchopleural fistula, empyema, or residual space requiring thoracoplasty occurred in the presence of a normal bronchial submucosa, while the 19 complications that developed in these patients were equally

TABLE 1

SUBMUCOSAL STUDY OF 320 RESECTED BRONCHI IN PATIENTS WITH TUBERCULOSIS

	Number of Patients	Complications			
		Broncho-pleural Fistula	Residual Space	Total	Per Cent
Normal	34	0	0	0	0.0
Lymphocytic infiltration	240	22	21	43	17.9
Tubercles	46	10	2	12	26.0
Total	320	32	23	55	17.2

distributed between those with submucosal lymphocytic infiltration and tubercle formation. Since the publication of these observations, the present writers have continued this study which now includes 320 surgical resections for pulmonary tuberculosis; 55 of the patients in this group developed postoperative complications. To date no complications have occurred in any of the patients with normal submucosa at the point of bronchial transection (table 1). Since the finding of this pathologic process within the bronchus following resection is of little practical value, the present study was carried out to establish whether preoperative biopsy of the bronchus leading to the area of pulmonary disease would reflect the condition of the bronchial submucosa as observed following resection.

[1] From the Ohio Tuberculosis Hospital; and the Division of Thoracic Surgery, Department of Surgery; and the Department of Pathology, Ohio State University Health Center, Columbus, Ohio.

[2] Presented before the Medical Session, as part of *Section 2A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

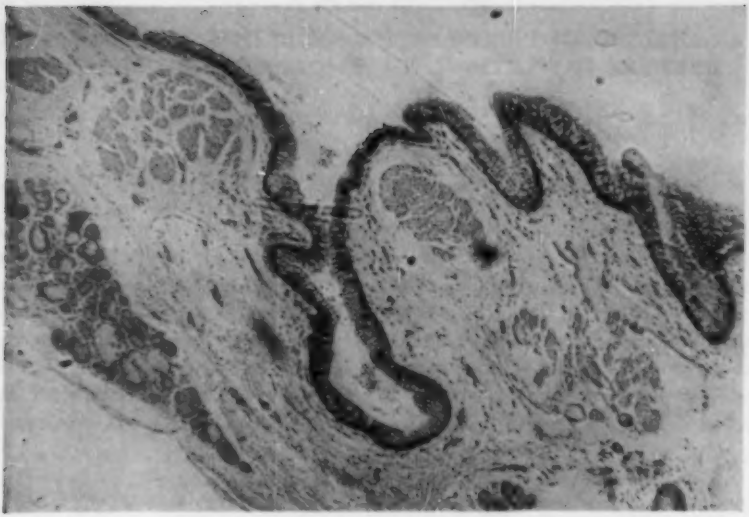


FIG. 1. Normal bronchus. Note the intact mucosa and the delicate submucosal stroma with widely scattered lymphocytes. Muscle bundles and mucous glands are present. (150 ×)

FIG. 2. Submucosal lymphocytic infiltration of bronchus. Note the intact mucosa and the moderately intense diffuse lymphocytic infiltration in the thickened submucosa. A bronchial artery and cartilage are present. (150 ×)

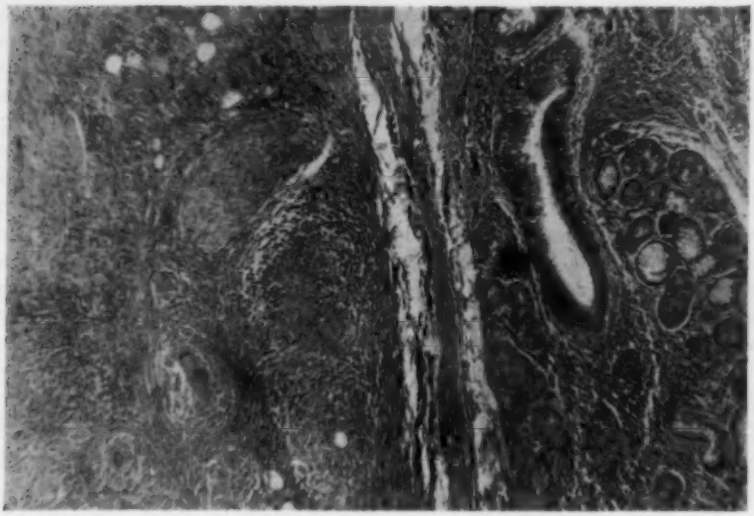


FIG. 3. Submucosal tuberculous bronchitis. Note the intense diffuse lymphocytic infiltration and the two tubercles at the left of center. The larger tubercle shows central caseation. These lesions are situated deep in the wall of the bronchus and the intact mucosa is not included. (150 ×)

MATERIALS AND METHOD

Between May, 1955, and December, 1956, 100 patients with pulmonary tuberculosis, in whom resection was contemplated, were bronchoscoped, and a biopsy was obtained from the carina of the bronchus supplying the area of intended resection. At the time of bronchoscopy a notation was made of the gross appearance of the tracheobronchial mucosa, especially at the site of bronchial biopsy. In most patients an interval of seven days was allowed before the resectional procedure was performed, although in some patients the delay was only two days. All patients were observed for at least three months following resection, and complications were recorded.

The complications considered to be specifically of bronchial origin were postoperative residual space, persistent bronchopleural fistula, or empyema. Residual space requiring thoracoplasty was included as a bronchial complication because, in at least some instances, this occurred as a result of bronchial failure. No empyema occurred in this group of patients without a concomitant bronchopleural fistula. The bronchial biopsy and a cross-section of the resected bronchus from the surgical specimen were studied histologically for the presence or absence of submucosal abnormalities. Findings were classified microscopically as normal bronchial submucosa (figure 1), lymphocytic infiltration (figure 2), or tubercle formation (figure 3). The gross appearance of the bronchus at bronchoscopy, the histologic appearance of the bronchial biopsy, and that of the resected bronchus were related to each other and to the incidence of postoperative complications.

RESULTS

Fifty-four men and 46 women comprised the patient material for this study. The age range was from 16 to 74 years, with an average of 39.6 years. The pul-

monary tuberculosis was far advanced in 69 patients, moderately advanced in 30, and minimal in one patient (table 2). All complications occurred in the patients with far advanced disease. Forty-five patients had sputum negative and 55 had sputum positive for tubercle bacilli by microscopy or culture during the three months preceding resection (table 3). Bacteriologic studies of the tubercle bacilli cultured from preoperative sputum or from the resected tissue in 56 patients demonstrated that 43 of these patients had organisms susceptible to the drugs being administered, and in 13 the bacilli were resistant. None of the complications occurred in patients with resistant organisms (table 4). Cavities were present in the resected specimens of 48 patients and closed disease was present in 52 patients (table 5). The extent of pulmonary resection in these patients was segmental resection in 42, lobectomy in 38, combined lobectomy and segmental resection in 13, and pneumonectomy in 7 patients (table 6).

The complications of bronchial origin in this series of 100 patients included residual space which required tailoring thoracoplasty in 3 patients and bronchopleural fistula associated with empyema in 4. Two of the 4 patients who de-

TABLE 2

RELATIONSHIP OF EXTENT OF DISEASE TO COMPLICATIONS

Extent of Disease	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Far advanced	69	4	3	10.1
Moderately advanced	30	0	0	0.0
Minimal	1	0	0	0.0

TABLE 3

RELATIONSHIP OF SPUTUM (3 MONTHS PRIOR TO RESECTION) TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Tubercle bacilli present	55	3	0	5.5
Tubercle bacilli absent	45	1	3	8.9

TABLE 4

RELATIONSHIP OF SUSCEPTIBILITY STUDIES OF RECOVERED TUBERCLE BACILLI TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Susceptible	43	3	1	9.3
Resistant	13	0	0	0.0
No organisms cultured	44	1	2	6.8

TABLE 5

RELATIONSHIP OF STATUS OF DISEASE (SPECIMEN) TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Cavity present	48	3	2	10.4
No cavity	52	1	1	3.8

TABLE 6

RELATIONSHIP OF EXTENT OF RESECTION TO COMPLICATIONS

Extent of Resection	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Segmental resection	42	2	0	4.8
Lobectomy	38	0	3	7.9
Combined lobectomy and segmental resection	13	2	0	15.4
Pneumonectomy	7	0	0	0.0

veloped bronchopleural fistula died as a result of this complication and constituted the only mortalities in this study. No complications occurred which could be attributed to the preoperative bronchial biopsy.

The gross appearance of the tracheobronchial mucosa at the time of bronchoscopy was considered by the endoscopist to be normal in 87 patients. Thirteen patients were reported to have erythema of the mucosa of the tracheobronchial tree. None were reported to have acute ulcerative bronchitis.

Histologically, the biopsy of the bronchus draining the area of parenchymal disease was found to be normal in 24 patients, to show lymphocytic infiltration of the submucosa in 73, and evidence of tubercle formation in 3. One patient with submucosal tubercles had ulceration of the mucosa demonstrated by biopsy that could not be seen by gross inspection at bronchoscopy.

Histologic study of the resected bronchus at the line of surgical transection demonstrated normal submucosa in 10 patients, lymphocytic infiltration in 71, and tubercle formation in 19. Three patients with submucosal tubercles also had mucosal ulceration.

Sixty-five of the 87 patients with normal tracheobronchial mucosa at bronchoscopy had either submucosal lymphocytic infiltration or tubercle formation disclosed by biopsy, and 78 had lymphocytic infiltration or tubercle formation in the resected bronchus. Of the 13 patients with erythema noted at bronchoscopy, 11 were found to have either lymphocytic infiltration or tubercles present in the submucosa disclosed by the bronchial biopsy, and 12 to have an abnormal bronchial submucosa when the resected bronchus was examined. No complications occurred among these 13 patients showing erythema of the bronchial mucosa by inspection at bronchoscopy (table 7).

TABLE 7

RELATIONSHIP OF BRONCHOSCOPIC APPEARANCE TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Normal	87	4	3	8.0
Inflammation	13	0	0	0.0

TABLE 8

RELATIONSHIP OF BRONCHIAL BIOPSY TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Normal	24	0	1	4.2
Lymphocytic infiltration	73	4	2	8.2
Tubercles	3	0	0	0.0

TABLE 9

RELATIONSHIP OF RESECTED BRONCHUS TO COMPLICATIONS

	Number of Patients	Complications		
		Bronchopleural Fistula	Residual Space	Per Cent
Normal	10	0	0	0.0
Lymphocytic infiltration	71	4	3	9.9
Tubercles	19	0	0	0.0

When the complications were related to the microscopic findings in the bronchial biopsy, it was found that one patient developed a residual space requiring thoracoplasty despite the finding of normal bronchial submucosa in the biopsy. The transected bronchus in this case showed lymphocytic infiltration. Six of the 73 patients showing lymphocytic infiltration developed either a residual space or a bronchopleural fistula, but none of the 3 patients with tubercles discovered by bronchial biopsy developed a complication (table 8).

When the resected bronchi were studied in relationship to postoperative difficulties, no complications occurred in the 10 patients with normal bronchial submucosa. All 7 complications occurred in patients showing lymphocytic infiltration, and none developed in the 19 patients showing tubercle formation in the bronchial submucosa (table 9).

DISCUSSION

As experience has been gained in the resectional treatment of pulmonary tuberculosis, certain factors influencing the postoperative development of complications have been recognized. It has been accepted generally that the pa-

tient with the more advanced stages of tuberculosis, with active disease, with sputum positive for tubercle bacilli, with organisms which are not susceptible to any of the drugs used in the treatment of tuberculosis, and with tuberculous bronchitis has a greater chance of developing postoperative complications than those patients in whom these factors are absent. Furthermore, it has been accepted that closure of the bronchus by a technique which does not disturb the bronchial blood supply, as well as pleuralization of the bronchial stump, aids in reducing the postoperative complications of bronchial origin. Yet, despite careful attention to these factors, complications of bronchial origin occur in patients in whom resection for this disease is carried out.

The observation has been made by Medlar (2) and by Olson and co-workers (3) that the bronchial submucosa may be abnormal in patients with pulmonary tuberculosis despite a normal-appearing mucous membrane. That this submucosal abnormality might be an unrecognized factor in the development of postoperative complications has been suggested (1). Support is lent to this concept by the submucosal study of 320 resected bronchi in patients with pulmonary tuberculosis in whom no complication has occurred in the presence of a normal submucosa. However, the finding of a bronchial abnormality after the surgical procedure has been accomplished offers little but statistical correlation. Only if this information is available prior to the time of resection can it be predicted that a patient might or might not develop a complication of bronchial origin. To study this, bronchial biopsy has been carried out as part of the preoperative study in 100 patients.

The bronchial biopsy did not reflect the true condition of the bronchial submucosa at the point of transection in all patients. Twenty-four patients were considered to have normal submucosa by biopsy while only 10 of these were found to have normal bronchial submucosa when the resected specimen was examined. It is not surprising that the bronchial biopsy should differ occasionally from the resected bronchus, inasmuch as the biopsy was obtained some distance proximal to the line of subsequent resection, especially when segmental resection was performed. In the patients treated by lobectomy or pneumonectomy, the biopsy reflected more accurately the submucosa in the resected bronchus. Despite this lack of complete correlation, the fact that only one patient with a normal bronchoscopic biopsy developed a residual space indicates the general reliability of the preoperative biopsy.

The absence of complications in the 19 patients who had tubercles in the submucosa of the resected bronchus deserves comment. It would appear that this can only be explained by chance distribution as, in the first 100 patients studied, the distribution of complications between those with lymphocytic infiltration and tubercles in the resected bronchus was identical; and in a more recent group the incidence of complications was much greater in those patients with submucosal tubercles. This observation is substantiated by table 1.

The observation that no complications occurred in the 13 patients with erythema of the tracheobronchial mucosa is of interest, but does not conflict necessarily with the concept of Hardy and Samson (4) that the patient with

acute tuberculous tracheobronchitis should not have surgical therapy. The erythema found in these patients does not correspond to the acute hyperemic, edematous, and often ulcerated tracheobronchial mucosa that has been accepted as a contraindication for resectional surgery. This erythema probably represents a nonspecific bronchitis or a mucosal reaction to the local anesthetic agent administered just prior to the performance of the bronchoscopic examination.

If the bronchial submucosa is a significant and additional factor in the development of postoperative bronchial complications in the patient with pulmonary tuberculosis, then efforts must be made to find some means of improving or converting this condition of abnormality to one of normality prior to resectional surgery. If this can be achieved, complications following resection should be reduced to an even greater extent than has been achieved in the past. Such a study is in progress.

Summary

Pre-resectional bronchoscopy and bronchial biopsy have been performed in 100 patients to determine whether this procedure can be done safely and will demonstrate the condition of the submucosa of the bronchus leading to an area of pulmonary tuberculosis. No complications have resulted from routine bronchial biopsy. A fairly accurate preoperative evaluation of the condition of the bronchial submucosa can be obtained by biopsy.

Inflammation of the bronchial submucosa appears to be related to the occurrence of post-resectional complications of bronchial origin. With a safe pre-resectional method of determining the condition of the bronchial submucosa by bronchial biopsy available, it is postulated that, if a method can be found to convert the condition of the bronchial submucosa from abnormal to normal prior to resection, the incidence of complications of bronchial origin can be further reduced.

Sumario

Análisis de la Biopsia Bronquial Preoperatoria en Cien Enfermos de Tuberculosis Pulmonar

En 100 enfermos se ejecutaron la broncoscopia y la biopsia bronquial antes de la resección para determinar si cabe llevar a cabo inocuamente este procedimiento y si revelará el estado de la submucosa del bronquio que conduce a una zona de tuberculosis pulmonar. No ha habido complicaciones a consecuencia de la biopsia bronquial corriente. Con la biopsia puede obtenerse una valuación preoperatoria bastante exacta del estado de la submucosa bronquial.

La inflamación de la submucosa bronquial parece relacionarse con la aparición de complicaciones postrreseccionales de origen bronquial. Contando con un método prerreseccional inocuo para determinar el estado de la submucosa bronquial, presupónese que, si puede descubrirse un método que convierta el estado de dicha submucosa de anormal en normal antes de la resección, cabe reducir aun más la incidencia de complicaciones de origen bronquial.

Resume

Une analyse de la biopsie bronchique préopératoire chez cent malades atteints de tuberculose pulmonaire

La bronchoscopie et la biopsie bronchique avant résection ont été effectuées chez 100 patients pour déterminer si cette méthode pouvait être pratiquée sans danger et si elle démon-

trerait l'état de la sous-muqueuse de la bronche aboutissant à une zone de tuberculose du poumon. La biopsie bronchique systématique n'a entraîné aucune complication. La biopsie permet une évaluation préopératoire assez exacte de l'état de la sous-muqueuse bronchique.

L'inflammation de la sous-muqueuse bronchique paraît en relation avec les complications d'origine bronchique survenant après la chirurgie d'exérèse. Disposant d'une méthode inoffensive permettant de déterminer par biopsie l'état de la sous-muqueuse bronchique avant résection, les auteurs estiment que si l'on découvrait un procédé permettant de ramener à la normale une sous-muqueuse bronchique altérée, avant de procéder à la résection, la fréquence des complications d'origine bronchique serait encore réduite.

REFERENCES

(1) Andrews, N. C., Pratt, P. C., Gilman, R. A., and Klassen, K. P.: The correlation of the pathology in the resected bronchus with postoperative complications in pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *74*, 874.

(2) Medlar, E. M.: The behavior of pulmonary tuberculous lesions, Am. Rev. Tuberc., 1955, *71* (Supplement, pp. 133–134).

(3) Olson, D. E., Jones, F. S., and Angevine, D. V.: Bronchial disease in lungs resected for pulmonary tuberculosis, Am. Rev. Tuberc., 1953, *68*, 657.

(4) Hardy, K. L., and Samson, P. C.: The "quiescent" tuberculous bronchus: A review with a study of its surgical connotations, Am. Rev. Tuberc., 1956, *73*, 451.

EXPERIMENTAL PULMONARY EMPHYSEMA[1]

Production of Emphysematous Bullae in the Rabbit by Infection with Tuberculosis

JOHN W. BELL

(Received for publication July 15, 1958)

Introduction

The study of chronic obstructive pulmonary emphysema in humans has been limited by the difficulty of producing this lesion in the experimental animal. Most reports of the morphologic anatomy of emphysema have been made in necropsy studies of patients with far advanced disease (1–3). Few studies are available describing the histology of the early or moderately advanced lesion. Since the latter material would be available chiefly from surgical specimens or accidental death, it is necessarily limited. With emphysema as a common and increasing problem in the elderly male, continued investigation is necessary to understand the multiple factors involved in its pathogenesis.

For some time, chronic endobronchial infection, specific or nonspecific, has been a major factor in the pathogenesis of bronchiolar fibrosis, narrowing and increased rigidity. Although the end result of such chronic bronchiolitis has been carefully described, such evidence is available only when an equivalent lesion can be produced in the experimental animal, allowing retrospective study of serial changes.

While working with the effect of antituberculous chemotherapy on experimental tuberculosis in the rabbit, Steenken and Wolinsky (4) observed the development of large pulmonary cysts or bullae in several of the infected animals. Since peripheral obstructive emphysema has frequently been observed in humans (5–7) in association with areas of pulmonary tuberculosis, it was the purpose of the present writer to study the role of this chronic infection in the genesis of obstructive emphysema in the rabbit.

Materials and Method

Twelve random rabbits, each weighing 4 to 6 pounds, were infected intratracheally with 0.25 ml. of a normal saline suspension containing 1.0 mg. (dry weight) of tubercle bacilli. The bacillus used was *M. tuberculosis* var. *bovis*, strains No. 4228–4 and No. 2128 (obtained from the Trudeau Laboratory) susceptible to streptomycin and PAS.

Chemotherapy was as follows: 150 mg. of streptomycin intramuscularly, daily; 1 gm. of PAS in 200 ml. of drinking water daily; 150 mg. of cycloserine twice a day intramuscularly. This regimen permitted 90 per cent of the animals to survive for various periods of time (table 1). Beginning at thirty days following inoculation, the animals were sacrificed or allowed to die when roentgenographic evidence of bullae was observed.

Vinyl acetate plastic corrosion specimens were made at autopsy on one or both lungs of 7 animals showing bullae or blebs. The plastic material flowed readily into the fine bronchial

[1] From the Department of Surgery, Veterans Administration Hospital, Seattle, Washington.

TABLE 1

EXPERIMENTAL EMPHYSEMA

Rabbit Number	Chemotherapy	Died After Infection	Blebs
		days	
305	Streptomycin and PAS	16	No
310	Streptomycin and PAS	19	No
308	Cycloserine	22	No
309	Cycloserine	24	No
307	Streptomycin and PAS	31	Yes
315	Cycloserine	39	No
304	Streptomycin and PAS	42	No
316	Streptomycin and PAS	56	Yes
312	Cycloserine	57	Yes
314	Streptomycin and PAS	65	Yes
317	Cycloserine	104	No
313	Cycloserine	119	Yes

radicals with minimal pressure on the injecting syringe. The specimens were corroded in 10 per cent potassium hydroxide and were studied and photographed after washing (figures 1–8). Because of their delicate nature they were later embedded in a block of clear casting plastic for preservation.

For study of the pathogenesis of this experimental lesion, the lungs of 3 rabbits that died or were sacrificed at intervals of thirty-one days (No. 307), fifty-six days (No. 316), and one hundred and nineteen days (No. 313) were inflated by gentle intrabronchial injection of 10 per cent formalin until they had attained a size equal to that of normal inspiration (figure 5). Both lungs of each rabbit were sectioned from base to apex in 25 blocks, each 4 to 5 cm. thick. Serial sections were cut from 15 to 20 micra. Every third section was stained with hematoxylin and eosin, and random sections were stained with elastic tissue stain. Approximately 450 sections per lung were examined serially.

RESULTS

Gross observations: It was determined that antituberculous chemotherapy should be instituted within one week of challenge; otherwise, nearly all animals died of tuberculous pneumonia. Rabbits infected by the intraperitoneal or intravenous route occasionally developed cystic pulmonary lesions similar to those seen with tracheal infection, but not with the consistency or degree following the latter method. Hematogenous dissemination, when it occurred, usually resulted in overwhelming disease, and the animals died despite chemotherapy.

Only those animals in which effective drugs permitted survival and resolution of the tuberculous pneumonia developed emphysema and bullae as a sequel to the infection. Weekly roentgenograms of the lungs were begun in the second week, as previous experience had shown that emphysematous bullae did not appear before thirty days post infection. The development of bullae could be observed quite well on serial films (figures 9–12).

At sacrifice the gross appearance of the rabbit lung was not unlike that of

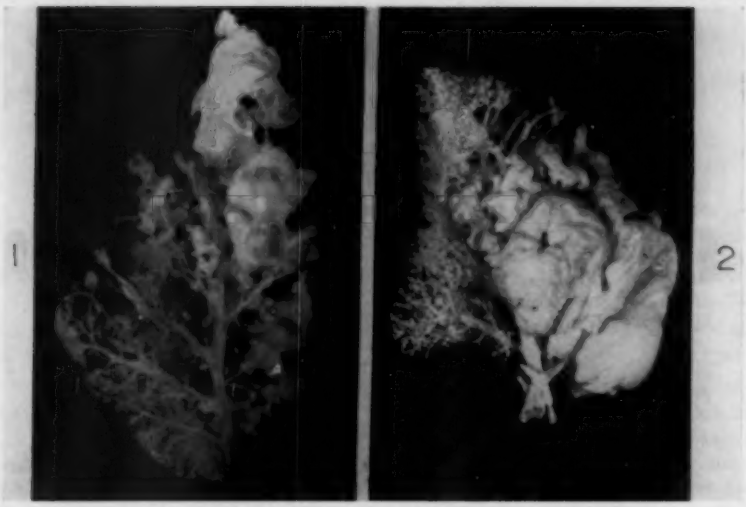


FIG. 1. Rabbit No. 316 died at 56 days. Vinyl acetate cast of lung showing multiple small and large peripheral blebs.

FIG. 2. Rabbit No. 312 died at 57 days. Vinylite cast showing large emphysematous bullae in dorsal lobe, relatively normal pattern in ventral lobes.

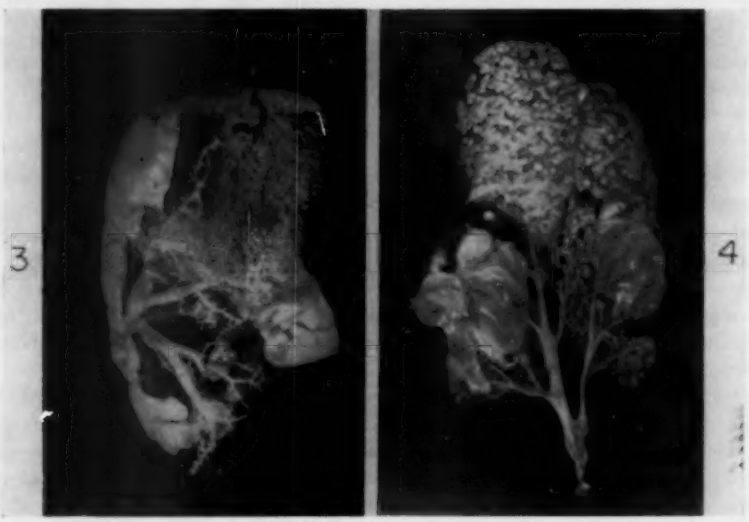


FIG. 3. Rabbit No. 313 died at 119 days. Vinylite cast showing enormous dorsal bulla but relatively normal pattern in remaining left lower lobe.

FIG. 4. Rabbit No. 328 died at 47 days. Vinylite cast showing moderate-sized peripheral blebs with multiple bronchocystic junctions.

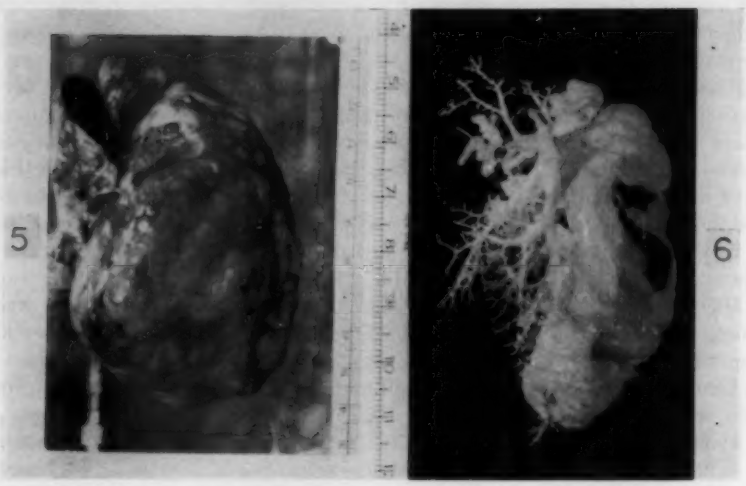


FIG. 5. Rabbit No. 314 died at 65 days. Gross specimen at time of inflation and fixation showing very large thin-walled peripheral bulla.

FIG. 6. Vinyl cast of same specimen revealing distortion of lung and loss of substance in area occupied by cyst.

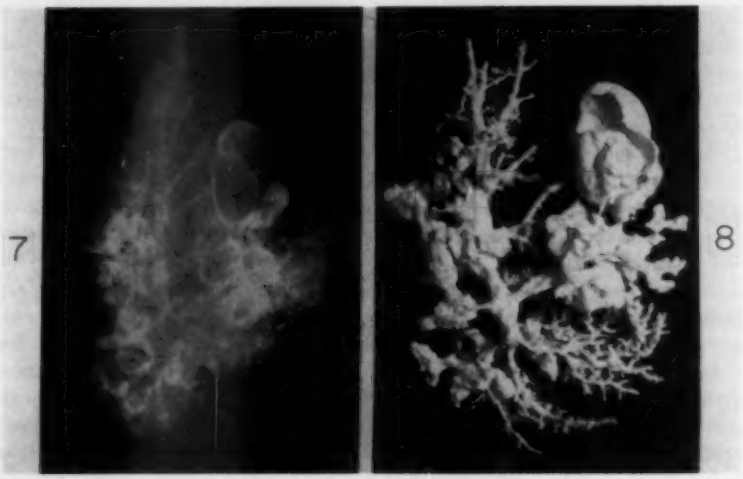


FIG. 7. Rabbit No. 307 died at 31 days. Bronchogram of fresh specimen showing multiple peripheral thin-walled blebs and arborization of bronchial tree.

FIG. 8. Vinyl acetate cast of same specimen showing egg-shell nature of blebs and origin from distal bronchi.

the lungs of humans with advanced tuberculosis. The bullae or cysts varied widely in size from the small peripheral bleb of several coalescent alveoli to the giant bulla replacing a lobe.

Most commonly, the area of emphysematous change was seen in the dorsal portions of the lobe corresponding to the apical location in the erect animal. There was a clear distinction between a tuberculous cavity and the obstructive bullae. The cavity was typically more central in the parenchyma, with a thick, dirty wall, and contained necrotic material. On the other hand, the bullae were peripheral, thin-walled, and often translucent (figures 5–8). They were seen to be covered with a layer of thin visceral pleura and always demonstrated a clean lining.

Microscopic findings: Believing that the development of these bullae was a function of time as well as of the degree of infection, representative sections from various lobes were studied in a serial manner and compared with the normal rabbit lung (figure 13). These will be described at intervals of seven, fourteen, seventeen, and thirty days after infection.

At one week, most sections revealed patchy to confluent homogenous areas of tuberculous pneumonia (figure 14). The interstitial tissues as well as the alveolar spaces were filled with an exudate rich in inflammatory cells. Many of the small and medium-sized bronchi were partially or completely filled with cellular exudate. The round cell infiltration included the perivascular spaces.

In the left lung of the rabbit sacrificed at fourteen days following inoculation, acute and subacute inflammatory changes predominated. Focal collections of round cells, mostly lymphocytes, were seen at bronchial bifurcations (figure 15). The peribronchial and perivascular spaces were filled with neutrophils and occasional giant cells. The lumens of many bronchioles were filled with amorphous material in which cellular debris was embedded. Further sections of the lower lobe revealed scattered areas of necrosis which were commonly in juxtaposition to the larger bronchi.

In several sections there was early organization with granulomatous formation. However, focal collections of cellular infiltration were so common in the peribronchial and perivascular spaces of the bronchus, artery, and vein that these could have been mistaken for early granulomas when their lumens were obliterated (figure 16).

Areas of necrosis appeared to extend into the peripheral alveolar septa. Whether this process contributed to septal disruption and subsequent coalescence of alveolar spaces, or whether the latter resulted from mechanical forces of increased inter-alveolar pressure, is uncertain. For the first time, areas of peripheral obstructive emphysema were observed. In repeated sections, many small bronchi and bronchioles could be traced as they led directly into coalescing alveoli or lacunar spaces (figures 17–19). The right lung showed marked periarterial thickening in both the pneumonic and nonpneumonic areas. The cellular element was so dense that the change resembled a panarteritis with involvement of all coats. The lumens of the vessel were often occluded with a colloid-like hydropic fluid.

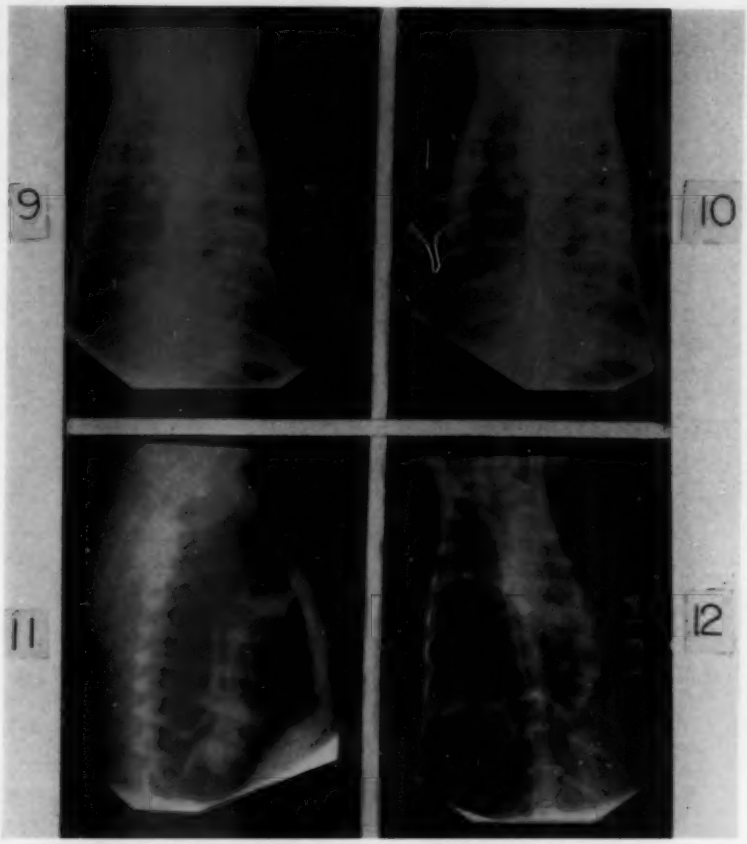


FIG. 9. Rabbit No. 314. Postero-anterior roentgenogram 36 days after infection showing right lower lobe bulla.

FIG. 10. Rabbit No. 314. Enlarging right lower lobe bullae 52 days after infection.

FIG. 11. Rabbit No. 314. Lateral roentgenogram showing multiple large bullae occupying right lower lobe.

FIG. 12. Rabbit No. 314. Enormous lower lobe tension cyst with mediastinal shift 60 days after infection and three days before death.

At seventeen days following injection, beginning resolution of the dense pneumonic infiltration was noted (figure 20). However, many of the small bronchi were filled with the products of exudation. Disruption of alveolar septa with formation of lacunae was common in the subpleural areas.

These changes were more advanced (figures 21, 22) from twenty-six to thirty days. Scattered areas of residual pneumonitis persisted in both lungs, more commonly in the upper or dorsal lobe. This patchy consolidation of intense

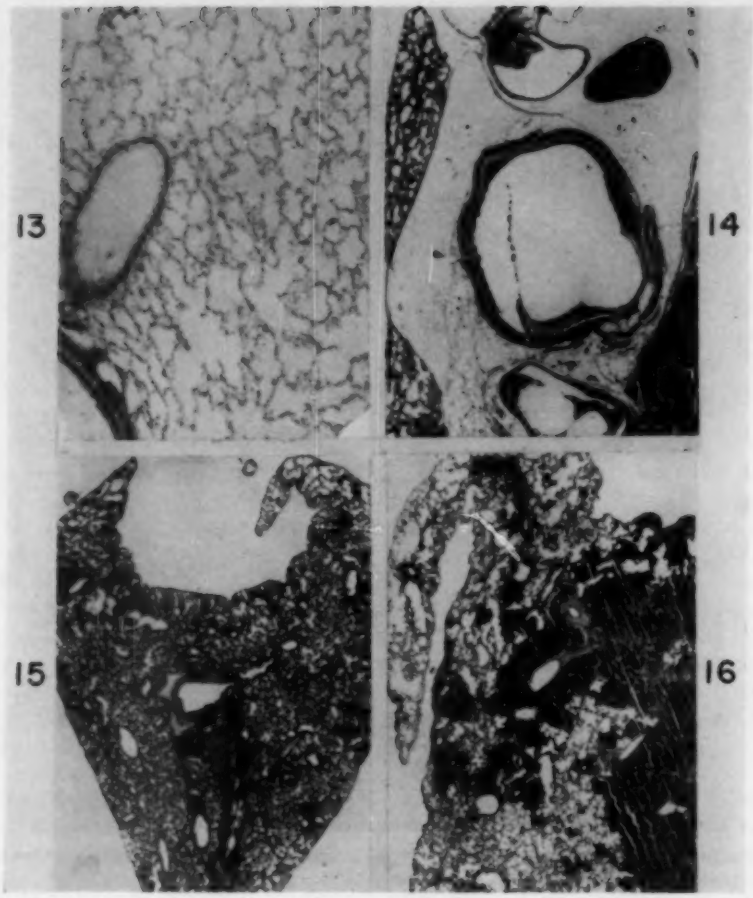


FIG. 13. Photomicrograph of normal rabbit lung showing alveolar pattern and medium-sized bronchi. (Hematoxylin and eosin stain, × 160)

FIG. 14. Right upper lobe section at 7 days after infection. Tuberculous pneumonia in margin. Cellular exudate in lumen of bronchi. (Hematoxylin and eosin stain, × 160)

FIG. 15. Left upper lobe section at 14 days. Collection of infiltrate at bronchial bifurcation. Focal collections of round cells in lumen of small bronchioles and peribronchial spaces. (Hematoxylin and eosin stain, × 160)

FIG. 16. Right upper lobe at 14 days. Periarterial inflammation in both pneumonic and nonpneumonic areas. Lumen thrombosed in many small vessels. (Hematoxylin and eosin stain, × 160)

round cell infiltration alternated with pre-emphysematous changes. The alveolar septa showed fragmentation and multiple coalescence of adjacent lacunae. These enlarging lacunae were either adjacent to bronchi or, more often, bronchi could be seen on serial review to enter directly into one corner of the air space.

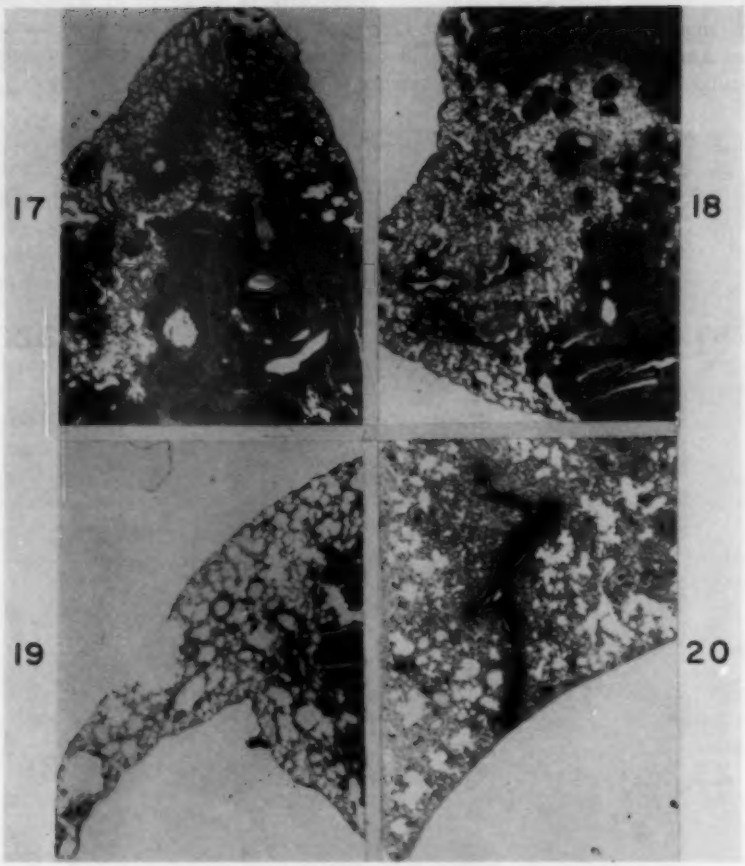


FIG. 17. Right lower lobe at 14 days. Scattered areas of necrosis with early granulomatous formation in the bronchovascular areas. Possible early lobular emphysema in the subpleural region. (Hematoxylin and eosin stain, × 160)

FIG. 18. Left lower lobe at 14 days after infection. Slight resolution of pneumonia. Cellular infiltration and necrosis of vessel and bronchial walls. Lumens partially or completely occluded. (Hematoxylin and eosin stain, × 160)

FIG. 19. Left lower lobe at 14 days. Peripheral section with coalescing alveoli and bronchioles leading into lacunae. (Hematoxylin and eosin stain, × 160)

FIG. 20. Left upper lobe at 17 days. A bronchus in longitudinal section filled with amorphous material. Respiratory bronchiole opening into lacunae. (Hematoxylin and eosin stain, × 160)

The last animal sacrificed at thirty to forty days (figures 23, 24) after challenge showed continued resolution of the tuberculous pneumonia. The left lung sections revealed that necrotic areas were giving way to focal granulomas. The latter were usually adjacent to bronchi and blood vessels.

The cellular morphology consisted principally of neutrophils, monocytes, and lymphocytes. Foreign body or Langhans' type of giant cells were uncommon. Amorphous material with cellular debris was scattered in many bronchovascular lumens. There was little evidence of fibrotic changes at this stage.

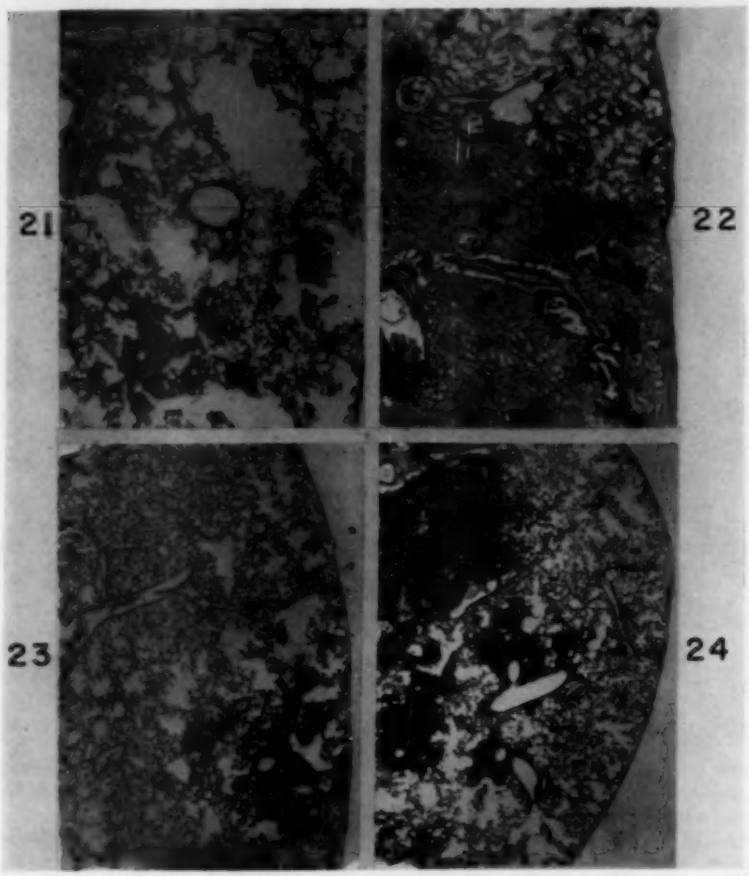


FIG. 21. Right lower lobe at 30 days. Pre-emphysematous changes. Enlarging lacunae with ruptured septa adjacent to central bronchus. (Hematoxylin and eosin stain, × 160)

FIG. 22. Left upper lobe at 30 days. Several medium-sized bronchi occluded with exudate and amorphous material. Partial resolution and early organization of pneumonia. (Hematoxylin and eosin stain, × 160)

FIG. 23. Left lower lobe at 30 days. Bronchus in longitudinal section. Scattered septal disruption with formation of lacunae. (Hematoxylin and eosin stain, × 160)

FIG. 24. Left lower lobe at 30 days. Pneumonic consolidation in left upper field. In lower center a small bronchiole may be seen leading directly into a subpleural lacunae or bleb. (Hematoxylin and eosin stain, × 160)

The essential histologic findings from seven to thirty days may be summarized. The major change at one week was the presence of an extensive tuberculous pneumonia. In the pneumonic areas the bronchial lumens were usually filled with inflammatory exudate. Some resolution of the pneumonia was apparent at fourteen days. Focal collections of round cells were seen at the bronchial bifurcation, the peribronchial, and perivascular areas. For the first time, possible pre-emphysematous changes were found. In many peripheral areas, often subpleural, small bronchi led directly into the coalescence of 3 or 4 alveoli. The latter small air spaces or lacunae might be the result of septal disruption from increased intra-alveolar pressure or actual necrotic destruction of the endothelium from the inflammatory process.

One of the most frequent histologic patterns was a broncholacunar junction in which a pseudocolumnar epithelium ended abruptly at the junction with an irregular space or enlarging alveolar air sac lined with a single layer of flattened epithelial cells. At this broncholacunar junction there was often a narrowing of the walls. This delta area commonly contained a plug of cellular or amorphous material.

One month after intratracheal infection, which was the earliest time the bullae were roentgenographically visible, the histologic precursor was found in the enlarging subpleural lacunae. The small bronchi and bronchioles were plugged with debris in the midst of pre-emphysematous changes. The latter were interpreted as enlarging lacunae and blebs resulting from alveolar septal rupture or disintegration.

Discussion

In the work herein reported, it was hoped to produce a subacute or chronic infection in the rabbit lung by modifying an acute tuberculous pneumonia with antituberculous drugs. The resolving infection would then tend to resemble the human lung with subacute or chronic bronchitis.

Both experimental and clinicopathologic evidence indicates that an inflammatory lesion crucially placed to obstruct air flow at the level of the respiratory bronchioles may produce emphysema. In the infant or child, this phenomenon is not uncommon in staphylococcal pneumonia.

Caffey (8) observed that tension pneumatoceles occurred most frequently in staphylococcal and other suppurative pneumonias, but were also seen in tuberculosis and similar granulomatous infections. He believed that these radiolucent areas constitute a special form of regional obstructive emphysema. The air cysts are believed to result from a check-value mechanism of intermittent obstruction by thick exudate, edema, and expiratory constriction in a small bronchus supplying an area of interstitial pulmonary sepsis. As the necrotic tissue is liquefied and evacuated through the bronchus, the resultant cavity is increasingly expanded with air.

Lindskog and Bloomer and associates (9, 10), having studied this condition clinically, believe there is a valving tension effect produced by obstruction from the bronchial edema and exudate and, at the same time, an interference

with collateral ventilation mechanisms in the peripheral portion of the pneumonitic lung.

Williams (11), in studying the etiology of asthma, bronchitis, and emphysema in children, found that bronchiolar spasm was often associated with acute or subacute infection of the bronchi. The inflammatory changes were often accompanied by the increased production of a thick, tenacious, mucinous exudate. The combination of edema, spasm, and increased mucus resulted in bronchial obstruction and, at least in the stage of incipient emphysema, "a ball valve mechanism" for the trapping of air in the terminal passages. He believed that repeated attacks of bronchopulmonary infection may lead to permanent obstruction with scarring and obliteration of the bronchiolar lumen.

Further observations on the role of infection were made by Campbell and his associates (13), who found suppurative bronchopulmonary disease to be one of the chief causes of acquired intralobar tension cysts. They noted the frequent roentgenographic appearance of pneumatoceles during the course of the bronchogenic spread of certain forms of interstitial pneumonia. These thin-walled air-containing cysts often enlarged rapidly and remained for many months after the pneumonic density had disappeared.

The late changes of emphysema have been studied by Amberson and Spain (14). They observed a chronic organizing constrictive bronchiolitis and peribronchiolitis with stiffening of the walls and narrowing of the bronchiolar lumens. These changes were usually seen in moderately or far advanced emphysema. In this state the bronchioles, both structurally and functionally, became quite insufficient (especially during expiration), thus operating as a check valve. It would appear that such a bronchiolar lesion is the end result of repeated bronchial inflammation with a chronic proliferative response. Spain and Kaufman (15) believe that a wide variety of low-grade irritants to the bronchiolar tree might be responsible for this nondestructive inflammatory process.

It is evident that pulmonary cavities have been produced mechanically (16), chemically (17–20), and by inflammatory processes. Whether air space disorders (21) produced experimentally or clinically by infection are evanescent or represent permanent destruction or distention of parenchyma is an important question. Indeed, Steenken and Wolinsky (4) observed in several rabbits that the cysts disappeared roentgenographically and were converted into linear scars. That such an end result occurs in human cystic pulmonary infections is well known.

Few of the rabbits were followed beyond a four-month period. The progressively enlarging air spaces and resolving tuberculosis led in most instances to death from pulmonary insufficiency. The ultimate fate of the bullae is uncertain. It is the impression, from a study of vinylite casts, that these pneumatoceles represent actual loss of pulmonary substance in addition to alveolar distortion.

The casting technique for the study of pulmonary architecture introduced by Loeschcke (22) and perfected by Liebow and associates (23) has demon-

strated communications among adjacent air spaces and multiple inosculations of bronchi with the bullae. Liebow states that a bulla represents far more than an overexpanded alveolus, and is rather the residue of a considerable mass of pulmonary substance.

Sandler (24) has studied the development of the tension cavity in pulmonary tuberculosis and believes the original lesion to be a small defect in the parenchyma resulting from the excavation of a necrotic focus. Subsequent enlargement of the cavity in the absence of a progressive caseating lesion is most likely due to a check-valve mechanism. The check valve may be due entirely to an endobronchial lesion which causes obstruction, or it may be due to extrinsic compression or distortion. This writer believes that the mechanism which can produce tension cavities in pulmonary tuberculosis can also produce them in any type of lesion that can cause focal parenchymal infiltration and an accompanying bronchial lesion. Bronchial obstruction occurring in asthma, chronic bronchitis, or any disease or disorder accompanied by bronchial narrowing (endobronchial or peribronchial) may also lead to air trapping and thus produce emphysema, cysts, or blebs.

In conclusion, although far advanced peripheral obstructive emphysema may be the same end result of different pathologic processes, as suggested by Paine (25), it is the impression of the present writer that chronic, intermittent, low-grade bronchial infection is a major contributing factor. Recognition and general acceptance of this concept, leading to the removal of external irritants and prompt aggressive treatment of pulmonary infection, may reduce the increasing morbidity and mortality of this lesion.

Summary

Changes of acute and subacute inflammation in the bronchioles of rabbits have been produced by the intratracheal injection of bovine tuberculosis.

Vasculitis and bronchiolitis with a peribronchial pneumonia have been seen as constant features in serial sections of lungs from rabbits sacrificed prior to the development of overt emphysematous bullae.

It would appear that the emphysematous process begins with tissue damage, expansion occurring subsequently. The fragmentation of tissue which is typical of centrilobular emphysema may depend upon weakening by inflammation. The organization of endobronchiolar and peribronchiolar exudate adds to the element of obstruction, and coalescent alveoli and lacunae enlarge in the subpleural areas where they are least well supported.

The pathogenesis of this lesion in the experimental animal appears to resemble the familiar lesion in the human with chronic bronchitis and hypertrophic emphysema.

Sumario

Enfisema Pulmonar Experimental. Producción de Bullas Enfisematosas en el Conejo por Infección con Tuberculosis

Por medio de la inyección intratraqueal de tuberculosis bovina se produjeron alteraciones de inflamación aguda y subaguda de los bronquiolos de los conejos.

Como característica constante se han observado vasculitis y bronquiolitis con neumonía peribronquial en los cortes seriados de los pulmones de conejos sacrificados antes de formarse vesículas enfisematosas declaradas.

Parece que el proceso enfisematoso comienza con una lesión de los tejidos, teniendo lugar la expansión después. La fragmentación del tejido, que es típica del enfisema centrilobular, tal vez proceda del debilitamiento por la inflamación. La organización de exudado endobronquiolar y peribronquiolar se sobrepone al elemento de oclusión y los alvéolos y lagunas coalescentes se agrandan en las zonas subpleurales, en donde encuentran menos apoyo.

La patogenia de esta lesión en el animal de experimentación parece semejar la conocida lesión en los seres humanos que tienen bronquitis crónica y enfisema hipertrófico.

Resume

Emphysème pulmonaire expérimental. Production de bulles emphysémateuses chez le lapin par inoculation de la tuberculose

L'inoculation intratrachéale de bacilles tuberculeux bovins a produit chez des lapins une réaction inflammatoire aiguë ou subaiguë des bronchioles.

L'angéite et la bronchiolite avec pneumonie péribronchique sont des signes constamment observés dans les coupes en série pratiquées dans les poumons des lapins sacrifiés avant le développement des bulles emphysémateuses caractéristiques.

Il semble que le processus emphysémateux débute par l'altération tissulaire, l'expansion se produisant ultérieurement. La fragmentation du tissu, caractéristique de l'emphysème centrolobulaire, pourrait dépendre de la perte de résistance causée par l'inflammation. L'organisation de l'exsudat endobronchiolaire et péribronchiolaire est un élément additionnel d'obstruction; les alvéoles coalescentes et les lacunes s'agrandissent dans les zones subpleurales où elles sont le moins tolérées.

La pathogénèse de cette lésion chez l'animal expérimental ressemble à la lésion familière rencontrée chez l'homme atteint de bronchite chronique et d'emphysème hypertrophique.

Acknowledgment

The writer is indebted to Joseph Koslouski, bacteriologist, Veterans Administration Hospital, Sunmount, New York, for his technical assistance in this study.

REFERENCES

(1) McLean, K. H.: The histology of generalized pulmonary emphysema, Parts I and II, Australasian Ann. Med., 1957, *6*, 124; 203.

(2) Hartroft, W. S.: The microscopic diagnosis of pulmonary emphysema, Am. J. Path., 1945, *2*, 889.

(3) Leopold, J. G., and Gough, J.: The centrilobular form of hypertrophic emphysema and its relation to chronic bronchitis, Thorax, 1957, *12*, 219.

(4) Steenken, W., Jr., and Wolinsky, E.: Formation of bullae or cystlike cavities during chemotherapy in rabbits with pulmonary tuberculosis, Am. Rev. Tuberc., 1957, *75*, 965.

(5) Alarcon, D. G.: Regressive giant bullous emphysema in tuberculosis of adults, Dis. Chest, 1955, *27*, 31.

(6) Bernonu, A., and Brun, J.: Cleansing of cavities and new formation of bullous cavities after chemotherapy, Presse méd., 1955, *63*, 894.

(7) Medlar, E. M.: Personal communication.

(8) Caffey, J.: Regional obstructive pulmonary emphysema in infants and children: Emphysematous cavities and their similarity to necrotic cavities, congenital pulmonary cysts and loculated pneumothorax, Am. J. Dis. Child., 1940, *60*, 586.

(9) Bloomer, W. E., Grammona, S., Lindskog, G. E., and Cooke, R. E.: Staphylococcal pneumonia and empyema in infancy, J. Thoracic Surg., 1955, *30*, 265.

(10) Lindskog, G. E.: In discussion of paper by Bloomer, W. E.

(11) Williams, S.: Aetiology and diagnosis of bronchial asthma, bronchitis, and emphysema in children, M. J. Australia, 1957, *44*, 782.
(12) Williams, S.: Bronchitis, asthma, and emphysema in childhood, M. J. Australia, 1957, *44*, 781.
(13) Campbell, J. A., Gastineau, D. C., and Velios, F.: Roentgen studies in suppurative pneumonia of infants and children, J.A.M.A., 1954, *154*, 468.
(14) Amberson, J. B., and Spain, D. M.: A mechanism explaining chronic progressive pulmonary bullous emphysema, Tr. A. Am. Physicians, 1947, *60*, 92.
(15) Spain, D. M., and Kaufman, G.: The basic lesion in chronic pulmonary emphysema, Am. Rev. Tuberc., 1953, *68*, 24.
(16) Moolten, S. E.: Mechanical production of cavities in isolated lungs, Arch. Path., 1935, *19*, 825.
(17) Yamamura, Y., Yasaka, S., Yamaguchi, M., Endo, K., Takeuchi, H., Nakamura, S., and Ogawa, Y.: Studies on the experimental tuberculous cavity: I. The experimental formation of the tuberculous cavity in the rabbit lung, J. Osaka University, 1954, *5*, 187.
(18) Yamamura, Y., Yasaka, S., Yamaguchi, M., Endo, K., Takeuchi, H., Nakamura, S., and Ogawa, Y.: Studies on the experimental tuberculous cavity: II. The experimental formation of tuberculous cavity in rabbit lung by killed tubercle bacilli, J. Osaka University, 1955, *6*, 197.
(19) Yamamura, Y., Yasaka, S., Yamaguchi, M., Endo, K., Takeuchi, H., Nakamura, S., and Ogawa, Y.: Studies on the experimental tuberculous cavity: III. The experimental production of tuberculous cavities in rabbit lung by an oil extract of killed tubercle bacilli, J. Osaka University, 1955, *6*, 479.
(20) Yamamura, Y., Yasaka, S., Nakamura, S., Yamaguchi, M., Ogawa, Y., Endo, K., and Takeuchi, H.: Experimental formation of tuberculous cavity by chemical constituents of tubercle bacilli, Proc. Japan Acad., 1955, *31*, 36.
(21) Rappaport, I., and Mayer, E.: Air space abnormalities of the lung, Am. J. Surg., 1955, *89*, 1002.
(22) Loeschcke, H.: Die morphologie des normalen und emphysematosen acinus des lunge, Beitr. path. Anat., 1921, *68*, 213.
(23) Liebow, A. A., Loring, W. E., and Felton, W. L.: The musculature of the lungs in chronic pulmonary disease, Am. J. Path., 1953, *29*, 885.
(24) Sandler, B. P.: Pathogenesis and treatment of pulmonary tension cavities, Am. Rev. Tuberc., 1957, *76*, 370.
(25) Paine, J. R.: Studies in the experimental production of pulmonary emphysema, J. Thoracic Surg., 1940, *10*, 150.

THE STABILITY OF THE TUBERCULIN REACTION[1]

SOLOMON KUNOFSKY AND JULIUS KATZ

(Received for publication February 10, 1958)

INTRODUCTION

Although there are many reports in the literature of the frequency of tuberculin conversion, there is little regarding the frequency with which tuberculin hypersensitivity is decreased or lost. The common impression seems to be that, once hypersensitivity is acquired, it is rarely lost except during some illnesses and perhaps in old age. In fact, the 1950 edition of the *Diagnostic Standards* of the National Tuberculosis Association states that "with few exceptions, definite sensitivity to tuberculin once acquired persists through life." It is the purpose of this paper to show that reversions of tuberculin sensitivity occur quite frequently and that there is a logical pattern to these changes.

MATERIALS AND METHODS

The data for the present report were obtained from a study of patients in two schools for mental defectives of the New York State Department of Mental Hygiene, the Newark State School and Letchworth Village.

In the Newark State School a study was made during the twelve-year period from 1936 to 1948 of the effect of exposure to infectious tuberculosis upon the subsequent development of this disease. All patients were tuberculin tested at the beginning of the study or on admission, and nonreactors were re-tested at six-month intervals. Reactors to tuberculin were not re-tested, since it was assumed at that time that reversions of the tuberculin reaction were infrequent. However, patients not reacting to tuberculin at the first test were re-tested at each of the subsequent semiannual testing periods even after tuberculin conversion. Unfortunately, shortly after the report of the original study was published (1), most of the records were destroyed. While the information considered necessary for that report had been transferred to punch cards which were retained, some of the data, which would have related the patients who showed changes in tuberculin reaction to the group as a whole, are not available.

However, the complete records of 411 patients who were originally tuberculin negative but who became reactors were retained. These patients, representing about 10 per cent of the total number of patients in the original study, are included in this report. The inability to relate this group to the entire population of the school does not affect the results of the study. They are not meant to be a representative sample of the school population, but are merely a portion of the school population who became tuberculin reactors and were re-tested frequently so that changes in tuberculin sensitivity could be observed. They were in no way selected for this study except for the retention of their records. They were Mantoux tested, using an intermediate dose of 0.00025 mg. of purified protein derivative (PPD). All testing and reading of the reactions were done by two physicians experienced in this type of work. Any recognizable induration was considered evidence of tuberculin sensitivity regardless of the extent, since the 1935 edition of *Diagnostic Standards* did not require the 5 mm. minimum specified in later editions. Reactions up to 10 mm. in diameter were classified as 1-plus, those 10 to 20 mm. as 2-plus, those more than 20 mm. as 3-plus, and the presence of necrosis as 4-plus.

---

[1] From the New York State Department of Health, Division of Tuberculosis Control, Albany, New York.

In Letchworth Village, patients were tuberculin tested in August, 1948, as part of a BCG vaccination program. They were Mantoux tested with first-strength PPD (0.00002 mg.) and nonreactors to this dose were re-tested with the second-strength dose (0.005 mg.). All of the 1,992 original reactors were re-tested in March and September, 1951 and 1952. These patients are the subject of the present report. Most of them were under observation during the entire four-year period. The shortest time between the first and last tuberculin tests was 2.5 years. The testing and reading of the tests were done by an experienced physician from August, 1948, to March, 1952, after which it was done by a registered nurse who was also experienced in this work. The tuberculin reactions were measured but, to simplify the utilization of the data for this report, the degree of reaction was translated into terms of 1 to 4-plus, as for the Newark group, except that a reaction of 5 mm. or less was considered negative.

There is some degree of error inherent in the interpretation of the tuberculin test whether the results are read in terms of 1, 2, 3 and 4-plus, or whether the induration is recorded in millimeters. These errors occur in the regions of transition between the negative and 1-plus reactions, and between the 1, 2, 3, and 4-plus groupings when the results are read in this manner, as well as in the precise determination of the point of demarcation between the induration and normal skin when actual measurements are recorded. The result at the lower end of the scale is the inclusion of some nonreactors in the reactor group or vice versa; while, among definite reactors, some may be placed in a group of lower intensity of reaction, others in a higher group.

These sources of error are so well known as to barely need mention. In order to remove their possible effect upon the results of this study, emphasis is placed mainly upon the changes among those with 2, 3, or 4-plus reactions. The possibility of reactions of this size being interpreted as negative, or of equivocal reactions being recorded as 2, 3, or 4-plus, is quite remote. Among those with 2-plus reactions or greater, the inclusion of some patients in a higher or lower category would not materially affect the results of the study, and it is quite probable that the incorrect inclusion of some patients in a higher category is compensated for by errors in the opposite direction in others.

Results

Newark State School

The data in table 1 show considerable variation in tuberculin sensitivity for the group as a whole, at different times during the study. Thus, although all patients were tuberculin positive at some time during the study (column 1 of the table), almost 41 per cent were negative at another time (column 2), while 15 per cent were negative at the end of the observation period. Similar variations were found at all levels of tuberculin sensitivity in the group.

The material in table 2 shows the variation in tuberculin sensitivity in more detail. The most extensive tuberculin reactions developed by the 411 original nonreactors and the lowest level of reaction after this peak had been reached are presented. Most tuberculin reactions did not remain static after reaching their peaks, but tended to decrease in extent with the passage of time. For example, the reaction in 20 (38 per cent) of 53 patients who developed 4-plus reactions subsequently reverted to negative, while, among 112 whose maximal reaction was 3-plus, the reaction in 50 (45 per cent) became negative, and so on. For the group as a whole, 41 per cent of the original nonreactors who became reactors reverted back to their original nonreactor status at some time during the period of observation.

This instability of the tuberculin reaction is well shown by a comparison of the group at the height of their tuberculin reactions with that at the end of the study, as shown in table 1. Thus, while 13 per cent of the original nonreactors developed 4-plus reactions at some time during the study, at the end of the study the percentage was less than 3. Even though every patient was tuberculin positive during some part of the period of observation, subsequently 41 per cent reverted to negative. Some of these in turn became positive again, so that only 15 per cent of all patients were tuberculin-negative at the end of the study.

The table shows that the distribution of the patients according to their tuberculin reactions at the end of the study provides no clue to the changes in sensitivity which had occurred previously.

TABLE 1

HIGHEST AND LOWEST TUBERCULIN REACTIONS AND REACTION AT END OF OBSERVATION PERIOD AMONG 411 ORIGINALLY TUBERCULIN-NEGATIVE PATIENTS IN NEWARK STATE SCHOOL

Tuberculin Reaction	Highest Reaction During Period (1)		Lowest Reaction During Period (2)		Reaction at End of Observation (3)	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Total	411	100.0	411	100.0	411	100.0
Negative	—	0	167	40.6	63	15.3
1+	100	24.3	152	37.0	202	49.1
2+	146	35.5	66	16.1	111	27.0
3+	112	27.3	15	3.6	24	5.8
4+	53	12.9	11	2.7	11	2.7

TABLE 2

HIGHEST TUBERCULIN REACTIONS OF ORIGINAL NONREACTORS, AND LOWEST REACTION SUBSEQUENTLY ATTAINED BY THEM
Newark State School

Tuberculin Reaction	Highest Reaction	Lowest Subsequent Reaction				
		No Reaction	1+	2+	3+	4+
		Number of Cases				
Total	411	167	152	66	15	11
1+	100	50	50	—	—	—
2+	146	47	52	47	—	—
3+	112	50	38	10	14	—
4+	53	20	12	9	1	11
		Percentage of Cases				
Total	100.0	40.6	37.0	16.1	3.6	2.7
1+	100.0	50.0	50.0	—	—	—
2+	100.0	32.2	35.6	32.2	—	—
3+	100.0	44.6	33.9	8.9	12.5	—
4+	100.0	37.7	22.6	17.0	1.9	20.8

Letchworth Village

All of the 1,992 patients in Letchworth Village included in this study were tuberculin reactors upon admission to the school. Because two strengths of PPD were used, a more detailed study of the changes in tuberculin sensitivity was possible. Among these patients, as in the Newark group, considerable fluctuation in tuberculin sensitivity was noted.

Among reactors to first-strength PPD, almost 8 per cent of those with 3-plus or higher reactions, 16 per cent of those with 2-plus reactions, and 44 per cent with 1-plus reactions became negative to this dose during the period of the study (table 3). If the end point of the tuberculin reaction is considered as failure to respond to second-strength PPD, the percentages are 4, 3, and 12, respectively.

Tuberculin reversions were much more frequent among those with lower degrees of tuberculin sensitivity, i.e., those originally sensitive only to the second dose of PPD. Here also the same trend was noted, that reversions were least frequent among patients with more marked reactions. Thus, about 31 per cent of those with 3-plus reactions reverted, as compared with 58 per cent among the 2-plus reactors, and 79 per cent among the 1-plus reactors (table 3). There appears to be a fairly well-defined pattern of reversion in tuberculin

TABLE 3

CHANGES IN TUBERCULIN REACTIONS AMONG LETCHWORTH VILLAGE PATIENTS

Reaction in the First Test	Total	Reaction in the Last Test							
		First Dose				Second Dose			
		3+	2+	1+	Negative	3+	2+	1+	Negative
Number of Cases									
First dose	1,387	115	798	216	258	25	150	18	56
3+ or more	115	*31*	65	10	9	1	4	—	4
2+	1,106	83	*689*	158	176	14	114	16	32
1+	166	1	44	*48*	73	10	41	2	20
Second dose	605	4	43	47	511	15	149	54	293
3+ or more	237	2	31	29	175	*10*	64	28	73
2+	330	1	11	18	300	5	*83*	22	190
1+	38	1	1	—	36	—	2	*4*	30
Percentage of Cases									
First dose	100.0	8.3	57.5	15.6	18.6	1.8	11.5	1.3	4.0
3+ or more	100.0	*27.0*	56.5	8.7	7.9	0.9	3.5	—	3.5
2+	100.0	7.5	*62.4*	14.3	15.9	1.3	10.3	1.4	2.9
1+	100.0	0.6	26.5	*28.9*	43.9	6.0	24.7	1.2	12.0
Second dose	100.0	0.7	7.1	7.8	84.5	2.5	24.6	8.9	48.4
3+ or more	100.0	0.8	13.1	12.2	73.8	*4.2*	27.0	11.8	30.8
2+	100.0	0.3	3.3	5.5	91.0	1.5	*25.2*	6.7	57.6
1+	100.0	2.6	2.6	—	94.7	—	5.3	*10.5*	78.9

The percentages in italic figures represent patients whose tuberculin reactions remained stable during the study.

sensitivity: the lower the degree of sensitivity, the more apt it is to revert completely to negative.

Although most emphasis is placed in this study upon changes involving decreases or loss of sensitivity, the unstable tuberculin reaction may, and often does, move in the other direction, toward an increase. These changes also progress from one stage to the next, as shown in table 3. The lower half of this table shows, percentage-wise, the relationship between the trends toward increases and decreases in sensitivity. The percentages underlined represent those patients whose tuberculin reactions remained stable during the study. Those to the left of the underlined figures showed increases in sensitivity; those to the right, decreases. It is obvious from the table that in this group the trend toward decrease in sensitivity is greater than toward increase. For example, almost 8 per cent of those responding to first-strength PPD with a 2-plus reaction at the initial testing showed an increase in sensitivity by a 3-plus or higher reaction at the final test, while in 30 per cent the last response was weaker than

TABLE 4

TUBERCULIN REACTIONS OF LETCHWORTH VILLAGE PATIENTS IN 1948, AND TYPE AND NUMBER OF CHANGES IN TUBERCULIN REACTION DURING THE SUBSEQUENT FOUR-YEAR PERIOD

Tuberculin Reaction in 1948	Total Tested	No Change	Continuous Trend		Multiple Changes	
			Upward	Downward	2	3 or more
Number of Cases						
First dose: 3+ or more	115	16	—	69	27	3
First dose: 2+	1,106	473	78	276	244	35
First dose: 1+	166	18	35	49	48	16
Total	1,387	507	113	394	319	54
Second dose 3+ or more	237	4	38	94	81	20
Second dose 2+	330	22	16	128	140	24
Second dose 1+	38	1	3	15	15	4
Total	605	27	57	237	236	48
Total, both doses	1,992	534	170	631	555	102
Percentage of Changes						
First dose: 3+ or more	100.0	13.9	—	60.0	23.5	2.6
First dose: 2+	100.0	42.8	7.1	25.0	22.1	3.2
First dose: 1+	100.0	10.8	21.1	29.5	28.9	9.6
Total	100.0	36.6	8.1	28.4	23.0	3.9
Second dose 3+ or more	100.0	1.7	16.0	39.7	34.2	8.4
Second dose 2+	100.0	6.7	4.8	38.8	42.4	7.3
Second dose 1+	100.0	2.6	7.9	39.5	39.5	10.5
Total	100.0	4.5	9.4	39.2	39.0	7.9
Total, both doses	100.0	26.8	8.5	31.7	27.9	5.1

the first. Among those with 1-plus initial responses, 27 per cent later showed more marked reactions, while 44 per cent showed less marked responses. The major trend among initial reactors to second-strength PPD was also toward a decrease in sensitivity.

The frequency with which changes in tuberculin reaction occurs is shown in table 4. During the four-year period of the study, only 37 per cent of patients sensitive to first-dose PPD maintained a fairly constant level of sensitivity, while only 5 per cent of those sensitive only to the second dose remained constant. The table also shows that changes in reaction did not always occur in the same direction. As shown in the last two columns, the direction of change is often first toward an increase in sensitivity and later a decrease, or vice versa. About one-third of the patients showed this irregularity.

Discussion

From the results of this study it appears that instability is one of the important characteristics of the tuberculin reaction. One of the major causes of this instability is probably the variation in exposure to infectious tuberculosis, with corresponding variations in the re-stimulating effect upon tuberculin sensitivity. Although the direct effect of an increase in the intensity of exposure in increasing the number of tuberculin reactors is seen very frequently, there is little information available regarding the effect of a reduction in exposure. Rich (2) mentions several reports of the loss of tuberculin sensitivity. He also points out that animal experiments show a disappearance of the tuberculin reaction in the absence of reinfection, and states that "it is reasonable to believe that a correspondingly larger number of individuals in the population today are escaping the hypersensitivity-reanimating influence of reinfections after having lost the sensitivity conferred by a primary infection." The original Newark study (1) demonstrated not only that a high prevalence of infectious tuberculosis was accompanied by a high incidence of tuberculin conversion, but also that a reduction in the prevalence of infectious tuberculosis was followed promptly by a similar reduction in the infection rate. In the present study, the downward trend in tuberculin sensitivity among the 411 patients in Newark State School coincided with a reduction in the incidence of tuberculosis in that institution (figure 1). When an upward trend in incidence occurred, a similar trend was observed for tuberculin sensitivity. Thus, higher conversion and lower reversion rates went with increased incidence, and vice versa.

The greater trend toward loss of tuberculin sensitivity among the patients in this study may be a reflection of the effect of the tuberculosis control program in the schools. This involves roentgenograms upon admission and periodically thereafter of all patients and employees, with prompt segregation of those with active tuberculosis, so that exposure to infectious tuberculosis is kept to a minimum. It is quite likely that under conditions of greater exposure, the major trend might be in the opposite direction, i.e., toward increase in sensitivity, as indeed did occur in the Newark group during those periods when the incidence of tuberculosis was high (figure 1).

Throughout the present study it was noted that the tuberculin reaction was

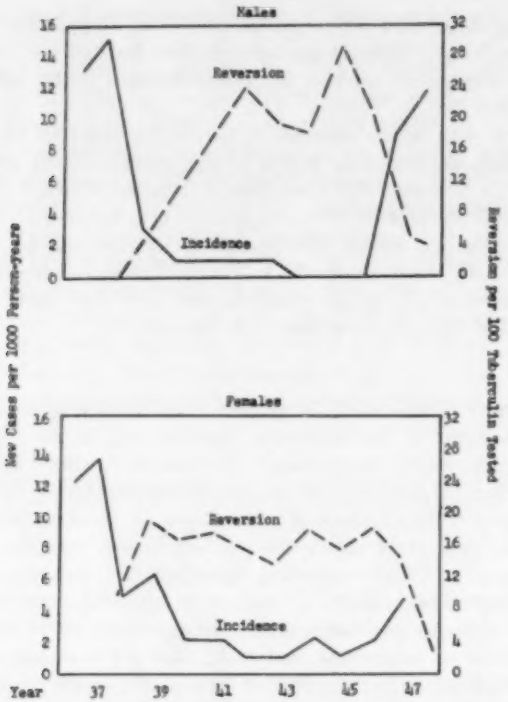


FIG. 1. Incidence of tuberculosis per 1,000 person-years and per cent of tuberculin reversions at the time of tuberculin testing for male and female patients of Newark State School.

most unstable among those who were least sensitive to tuberculin, i.e., those who reacted only to the second dose. Since the degree of sensitivity of these patients is so low, it may be assumed that changes in intensity of exposure become more readily manifest than among those with greater sensitivity.

In addition to the factor of exposure, there are, undoubtedly, other causes (individual or environmental in nature), such as severe illness (2, 3), which affect the tuberculin reaction. It may be changes of this kind which produce increases in sensitivity in some patients even though the trend for the entire group is toward reduced sensitivity, or vice versa. In this study the probability of such changes was reduced by not testing patients with severe illness.

While the environmental conditions under which the patients in the study lived are unusual, and the reversion rates may differ considerably from those which occur in the general population, there is no obvious reason to assume that instability of the tuberculin reaction does not exist in the general population. Some evidence that it does occur is provided by England, Muir, and Reynard

(4), who, in serial tuberculin testing (Heaf) a group of 504 industrial workers in England, found that 4 per cent of those positive in the first test were negative in the second a year later. Calculation of their data indicates that 3 per cent of those positive in the second test were negative in the third. These writers also found that "the trend among those with second degree reactions or higher was for the subsequent reactions to be *less* intense." This group is more representative of the general population and probably less subject to extremes of exposure than patients in the schools observed in this study; yet the types of tuberculin changes occurring are similar. It is probable that the concept of the constancy of the tuberculin reaction arose in the past when infectious tuberculosis was much more prevalent and exposure more frequent, so that sensitivity was maintained at relatively high levels. As the incidence and prevalence of tuberculosis continue to decline and opportunities for exposure to infectious tuberculosis are reduced, with the resulting reduction in the stimulating effect upon the tuberculin reaction, tuberculin reversion may become increasingly important.

It has been suggested that reactions to the stronger dose of tuberculin may be nonspecific (5). If this is so, it should strengthen the evidence for tuberculin instability since responses to second-strength PPD could be disregarded; in the Letchworth group, 19.6 per cent of the reactors would be considered reverters, rather than 4 per cent (table 3). Whatever the criterion may be, there is no doubt that loss of tuberculin sensitivity occurs, at least in some population groups and under certain environmental conditions.

The instability of the tuberculin reaction has an important bearing upon the interpretation of the results of tuberculin test surveys in that it presents a picture of conditions existing in individuals or groups only at the time of the survey, and one which is not necessarily representative of conditions prevailing previously.

Summary

A group of patients was tuberculin re-tested over a considerable period of time. It was found that the tuberculin reaction was quite unstable and often disappeared. One factor contributing to this was the change in exposure to tuberculous infection. Tuberculin conversions tended to increase, and reversions decrease, with increase in exposure. Both conversion and reversion are usually gradual processes. Obviously, this has an important bearing upon the interpretation of the results of tuberculin test surveys.

Sumario

La Estabilidad de la Reacción a la Tuberculina

Un grupo de enfermos fué recomprobado con tuberculina durante un período considerable de tiempo, observándose que la reacción producida era bastante inestable y a menudo desaparecía. Un factor que contribuyó a esto fué el cambio en la exposición a la infección tuberculosa. Los cambios en la reacción tendieron a aumentar y los virajes a disminuir, al aumentar la exposición. Ambos fenómenos suelen ser procesos graduales. Manifiestamente, esto guarda una relación importante con la interpretación de los resultados de las encuestas con pruebas tuberculínicas.

Resume

La stabilité de la réaction à la tuberculine

Un groupe de patients a été re-testé à la tuberculine pendant une période de temps considérable. On a découvert que la tuberculino-réaction était non seulement très instable, mais qu'elle disparaissait fréquemment. Une variation dans l'exposition à l'infection tuberculeuse constituait un facteur adjuvant. Les virages à la tuberculine avaient tendance à s'accroître et les réactions négatives à diminuer lorsque l'exposition à l'infection augmentait. Virage et cuti négative sont habituellement des processus graduels. Il est évident que ceci influe d'une façon importante sur l'interprétation des résultats des enquêtes basées sur le test à la tuberculine.

REFERENCES

(1) Plunkett, R. E., Katz, J., Thompson, M., and Donk, R.: Tuberculosis in a school for mental defectives, Am. J. Pub. Health, 1949, *8*, 1016.

(2) Rich, A.: Pathogenesis of Tuberculosis, ed. 2. Charles C Thomas, Springfield, Illinois, 1950.

(3) Furcolow, M. L., Emge, M. E., and Bunnell, I. L.: Depression of tuberculin and histoplasmin sensitivity associated with critical illness, Pub. Health Rep., 1948, *63*, 1290.

(4) England, N. J., Muir, J. M., and Reynard, W. A.: Serial tuberculin tests in adults, Tubercle, 1957, *38*, 213.

(5) Palmer, C. E.: Tuberculin sensitivity and contact with tuberculosis, Am. Rev. Tuberc., 1953, *68*, 678.

A FIVE-YEAR PILOT STUDY OF TUBERCULIN TESTING IN HONOLULU SCHOOLS[1]

Progress Report of the First Two Years of Observation

ROBERT H. MARKS, GEORGE TOKUYAMA, AND AGNES PETERSON

(Received for publication May 15, 1958)

INTRODUCTION

A five-year pilot study in tuberculin testing was begun in October, 1955, in certain public and private schools on Oahu, which is the island of location of Honolulu, the capitol and largest city of the Territory of Hawaii. The schools chosen were in the contiguous census tracts (tracts 2 through 11, 13, 16, 17) in Honolulu, which show the highest tuberculosis incidence rate, and included 23,000 of the 97,000 total school population on this island (figure 1). The present paper will briefly describe how the project developed and was organized. It will list the objectives, describe the techniques and methods used, and give the findings and impressions of two years' experience with this five-year project.

DEVELOPMENT AND ORGANIZATION OF THE PROJECT

Those concerned with tuberculosis control in the Territory had for some time been considering how to use the tuberculin test most successfully in the total control program. In 1953, with the encouragement of interest from pediatricians in the Honolulu area, and after the Coordinating Committee for School Health (an interdepartmental group from the Territorial Department of Health and the Department of Public Instruction) had approved of the project, a group of representatives from many agencies was called together by the Oahu Tuberculosis and Health Association to develop the prospectus. Participating were the Department of Public Instruction, the Honolulu County Medical Society, Leahi Hospital (the local tuberculosis hospital), the Territorial Department of Health (Bureau of Tuberculosis, Public Health Nursing, Health Statistics, and Office of Health Education), and others. When plans were formulated, a representative of the Oahu Tuberculosis and Health Association met with officials and teachers of the schools involved to explain the purpose of the testing, methods to be used, and the resulting follow-up study of positive reactors.

The five-year study is a joint enterprise of the Department of Public Instruction, the Bureau of Tuberculosis of the Department of Health, and the Oahu Tuberculosis and Health Association. The chief of the Bureau of Tuberculosis acts as medical director of the project. The Department of Health also furnishes chest clinic follow-up services and some public health nursing services for follow-up investigation outside the project area. The Oahu Tuberculosis and Health Association is the administrative agency and finances the study with funds raised by the annual sale of Christmas Seals. The cost for the first year was $28,400.

Objectives

The main objectives and some of the questions raised by these objectives are:

1. To determine how tuberculin testing surveys in the schools can be adapted to the future public health program for tuberculosis control. If a "positive" re-

[1] From the bureaus of Tuberculosis and Health Statistics, Territorial Department of Health, Honolulu, Hawaii; and the Tuberculin Testing Study, Oahu Tuberculosis and Health Association.

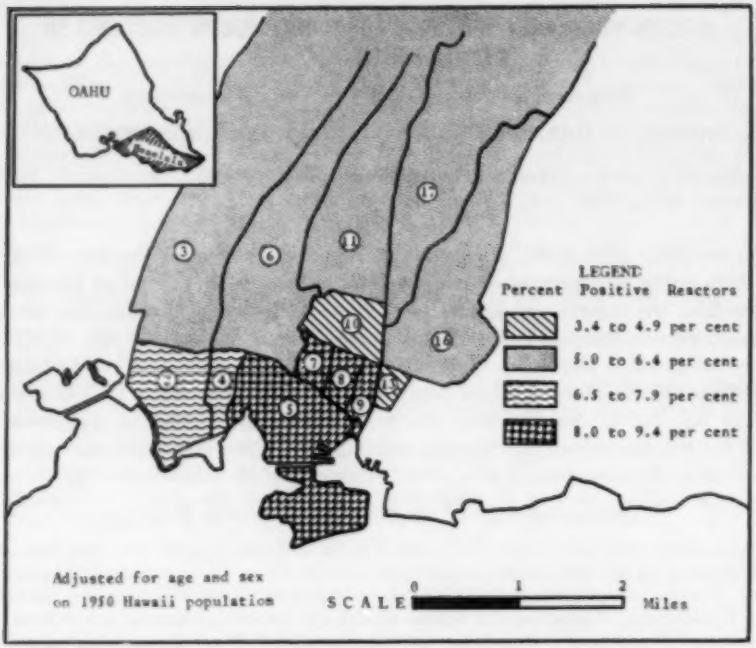


FIG. 1. Tuberculin sensitivity among children five to fifteen years of age in schools in selected census tracts, city of Honolulu, November, 1955, to September, 1956.

action to tuberculin indicates tuberculous infection, is it possible to locate the source of that infection, especially if the reactor is a child or if the reaction is known to have recently changed from negative to positive?

How effective is a routine tuberculin testing program in the school population as a means of finding new and unknown cases among those children who react and among the close associates of those children who react?

Will the yield of new cases discovered in the search for the source of a positive reaction in a child and in the examination of the child reactor himself make the expense of such a program feasible?

2. To reach a conclusion concerning the size of the reaction (in mm. of induration) which, in this community, will reveal most of those who are infected; yet will not include a high proportion of "false positives" or uninfected persons. If the lower limit of the size of the reaction which should be considered as a "positive" reaction is set too large, many truly infected persons would be missed. If it is set too small, it will include too many who are not infected.

3. To determine the prevalence of tuberculous infection in the study area. What is the extent of the tuberculosis problem in that area? The results of the tuberculin testing study added to that of the roentgenographic surveys and the information from the case register should give a good indication of the prevalence of tuberculous disease.

4. To determine the feasibility of "follow-up" of those children with positive reactions for a long period of time.

Are the children in this group, as opposed to those who are classed as nonreactors, the ones more likely to develop tuberculosis in the future?

Is this the group toward which the present case-finding efforts should be directed, rather than to the whole population?

Techniques and Methods

The testing and reading techniques and the equipment being used are those recommended in the manual, *Techniques of Tuberculin Testing and BCG Vaccination*, U. S. Department of Health, Education, and Welfare, Public Health Service. The test dose is 0.0002 mg. (10 tuberculin units), given in 0.1 ml. of solution. The tuberculin in use is Sharpe and Dohme tuberculin PPD tablets, and it is prepared freshly each testing day. Plastic metric rulers are used for measuring induration.

Two weeks prior to testing, a stenographer visits each school to organize cards and records. On testing day a team of two public health nurses and one practical nurse sets up the equipment and gives the tests. Volunteer mothers assist in cleansing the arms, stamping and arranging cards and consent slips, and keeping students in orderly lines. On reading day, two, three, or four days later (usually three days), public health nurses read the tests by classroom groups. Recording of results is handled by the project's clerical staff. In order to reduce the number of unread tests, home visits are made to as many as possible of the children who are absent on reading day.

Observations

Prevalence of Reactors

In the first year, the tuberculin reaction was determined for 94 per cent of the children enrolled in twenty-seven public and private Honolulu schools, or 22,958 of a total enrollment of 24,516 youngsters. Of these, 1,666 children (7.3 per cent) showed a reaction producing an induration with a transverse diameter of 6 mm. or more. During the second year of testing, 92 per cent of the children enrolled in the same public and private schools in Honolulu, or 23,993 of a total enrollment of 25,976 students, were likewise studied. Of these, 1,892 children (7.9 per cent) showed a reaction producing an induration with a transverse diameter of 6 mm. or more (table 1).

Current school records no longer include racial origins. Accordingly, racial origin as stated in the report is based on the racial identification supplied by the child or his family. Children of mixed ancestry which included Hawaiian forebears were included in the part-Hawaiian group, whatever the other ancestry. The results by ethnic group and sex for the first year of testing may be seen in table 2. Of the major groups the Japanese appeared to have the lowest prevalence (5.7 per cent for males and 4.7 per cent for females), and the Filipinos the highest (14.5 per cent for males and 12.0 per cent for females).

The prevalence of "positive" reactors by age and sex for two years of testing is set forth in figure 2. There appears to be a sharp increase in the prevalence of "positive" reactors in the male group after age fourteen and in the female group at age seventeen.

Although comparison with other tuberculin testing surveys is difficult because of the lack of uniformity in interpretation and techniques, there does appear to be some evidence that the prevalence of infection is greater here than in most areas of the mainland. The report of Palmer and associates (1) on the tuberculin

TABLE 1

AGE SPECIFIC TUBERCULIN-POSITIVE RATES IN SELECTED HONOLULU SCHOOLS BY SEX, BY SCHOOL YEAR, NOVEMBER, 1955, TO AUGUST, 1957

	1955–1956 School Year									1956–1957 School Year								
	Total			Male			Female			Total			Male			Female		
	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive
Total	22,958	1,666	7.3	11,366	934	8.2	11,592	732	6.3	23,993	1,892	7.9	11,826	1,042	8.8	12,167	850	7.0
Years																		
Less than 5*										203	3	1.5	109	2	1.8	94	1	1.1
5*	1,940	41	2.1	968	20	2.1	972	21	2.2	2,028	54	2.7	1,006	21	2.1	1,022	33	3.2
6	2,293	64	2.8	1,156	25	2.2	1,137	39	3.4	2,381	99	4.2	1,185	50	4.2	1,196	49	4.1
7	2,289	83	3.6	1,142	44	3.9	1,147	39	3.4	2,321	109	4.7	1,174	51	4.3	1,147	58	5.1
8	2,331	94	4.0	1,174	48	4.1	1,157	46	4.0	2,313	146	6.3	1,155	69	6.0	1,158	77	6.6
9	2,196	123	5.6	1,075	64	6.0	1,121	59	5.3	2,333	146	6.3	1,170	79	6.8	1,163	67	5.8
10	2,206	152	6.9	1,092	81	7.4	1,114	71	6.4	2,179	183	8.4	1,058	95	9.0	1,121	88	7.9
11	2,185	191	8.7	1,089	107	9.8	1,096	84	7.7	2,156	191	8.9	1,073	104	10.0	1,083	87	8.0
12	2,149	165	7.7	1,063	96	9.0	1,086	69	6.4	2,080	203	9.8	1,025	115	11.2	1,055	88	8.3
13	1,622	150	9.2	803	85	10.6	819	65	7.9	2,035	194	9.5	975	111	11.4	1,060	83	7.8
14	1,150	117	10.2	549	59	10.7	601	58	9.7	1,445	168	11.6	675	96	14.2	770	72	9.3
15	883	144	16.3	417	91	21.8	466	53	11.4	817	101	12.4	396	62	15.7	421	39	9.3
16	745	131	17.6	342	79	23.1	403	52	12.9	805	127	15.8	390	78	20.0	415	49	11.8
17	709	132	18.6	344	84	24.4	365	48	13.2	685	104	15.2	311	62	19.9	374	42	11.2
18	175	51	29.1	107	36	33.6	68	15	22.0	156	39	25.0	87	30	34.5	69	9	13.0
19 and older	73	27	37.5	39	15	38.5	34	12	35.3	53	22	41.5	36	16	44.4	17	6	35.3
Age unknown	12	1	—	6	0	—	6	1	—	3	3	—	1	1	—	2	2	—

* These two age groups were combined in the first year of testing.

testing of white Navy recruits (seventeen to twenty-one years of age) from metropolitan areas in 1949 to 1951 showed a 10.2 per cent prevalence of reactions of 5 mm.-plus to a 0.0001 mg. (5 TU) dose of PPD; whereas, in the first year of the present study, 25.1 per cent of 793 males aged sixteen through eighteen reacted

TABLE 2

FIRST-YEAR TUBERCULIN TEST RESULTS BY ETHNIC GROUP AND SEX FOR SELECTED HONOLULU SCHOOLS, NOVEMBER, 1955, TO SEPTEMBER, 1956

Ethnic Group	Male		Female	
	Number Tested	Per Cent Positive	Number Tested	Per Cent Positive
All groups	11,366	8.2	11,592	5.3
Hawaiian	164	11.0	182	11.0
Part Hawaiian	2,869	8.9	2,964	6.5
Puerto Rican	363	7.4	337	4.5
Caucasian	868	6.9	872	4.8
Chinese	1,113	9.1	1,203	7.1
Japanese	4,420	5.7	4,567	4.7
Korean	216	12.5	221	5.0
Filipino	1,288	14.5	1,178	12.0
Other groups	58	*	60	*
Group not stated	7	*	8	*

* Percentage not calculated because of small numbers.

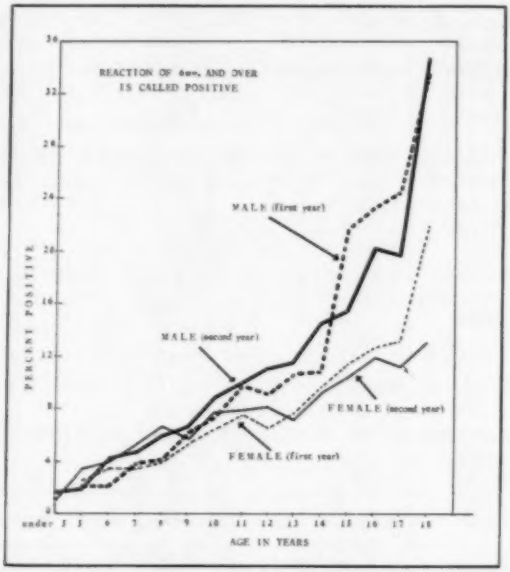


FIG. 2. Positive reactors in first- and second-year tuberculin testing by sex and age in selected Honolulu schools, November, 1955, to August, 1957.

to 0.0002 mg. (10 TU) with a 6 mm.-plus induration. In the second year, 21.6 per cent of 788 males in this age group had a reaction of 6 mm. or more. Mass tuberculin testing which has been done through the World Health Organization BCG programs appears to show little, if any, difference in the results when using 5 TU (0.0001 mg.) or 10 TU (0.0002 mg.) (2). It is possible, however, that a reaction of 6 mm. induration to 0.0002 mg., as used in the present study, corresponds roughly to a reaction of 5 mm. induration to the 0.0001 mg. dose, as used in Palmer's study. Therefore, it would appear that, in this section of Honolulu at least, the prevalence of infection is considerably higher than it is in continental United States.

The five-year average death rate from tuberculosis in this study area (1952–1956) was 10.7 per 100,000 (3), while the average tuberculosis death rate in

TABLE 3

CASES OF TUBERCULOSIS FOUND IN EXAMINING HOUSEHOLD ASSOCIATES OF TUBERCULIN-POSITIVE CHILDREN IN SELECTED HONOLULU SCHOOLS

November, 1955–August, 1957

Size* of Reaction in Index Child	Household Contacts		Cases of Tuberculosis									
			Active				Inactive				Total	
			New		Known		New		Known			
	Age Group	Number Examined	Number	Per Thousand	Number	Per Thousand	Number	Per Thousand	Number	Per Thousand	Number	Per Thousand
6–7 mm. only	15 years and older	2,338	—	—	4	1.7	—	—	35	15.0	39	16.7
	Less than 15 years	2,027	—	—	1	0.5	—	—	—	—	1	0.5
	Total	4,365	—	—	5	1.1	—	—	35	8.0	40	9.2
8 mm. and more	15 years and older	2,330	9	3.9	10	4.3	1	0.4	120	51.0	140	60.1
	Less than 15 years	1,577	—	—	—	—	—	—	18	11.4	18	11.4
	Total	3,907	9	2.3	10	2.6	1	0.2	138	35.3	158	40.4
Reverters*	15 years and older	732	—	—	—	—	—	—	9	12.3	9	12.3
	Less than 15 years	621	—	—	—	—	—	—	—	—	—	—
	Total	1,353	—	—	—	—	—	—	9	6.6	9	6.6
Total positive (6 mm. or more) on initial test	15 years and older	5,400	9	1.7	14	2.6	1	0.2	164	30.4	188	34.8
	Less than 15 years	4,225	—	—	1	0.2	—	—	18	4.3	19	4.5
	Total	9,625	9	0.9	15	1.6	1	0.1	182	18.9	207	21.5

* Children with reactions of 6 mm. and more in the first year who reverted to less than 6 mm. the second year.

continental United States during this period was 10.8 per 100,000 (4). The rate of new active and probably active cases reported in 1956 in this study area was approximately 87.5 per 100,000 as compared with the new case rate for the United States for 1956 of 41.2 per 100,000 (4). With this comparatively low death rate yet high prevalence of infection, one might be justified in concluding that the factors, whether spontaneous or applied, which affect tuberculosis in any area must be operating at a relatively high level in this community.

Yield of New Cases

In August, 1957, after two full years of the project, 8 new active cases of tuberculosis were found in 1,912 children who reacted to 10 TU with an induration of 6 mm. and more on their initial tests. During this period, 28,544 different school children were tested. This total included 22,958 tested during the first year of testing and 5,586 tested for the first time during the second year of testing. The yield was 0.3 per 1,000 school children tested.

The yield of new cases through examination of 9,625 household contacts (all ages) of children with "positive" reactions are shown in table 3. Nine new active cases of tuberculosis were discovered, giving a rate of 0.9 per 1,000 household associates for all ages examined. Since these 9 new cases of tuberculosis were found entirely in the household associates fifteen years of age and older, the yield from examining this group would be 1.7 per 1,000. The yield of new active cases of tuberculosis in the mass roentgenographic program conducted on Oahu for 1956 was 1.6 per 1,000 persons examined (5).

All of the 9 new cases of tuberculosis were found by examining 2,330 persons fifteen years of age and older who were household associates of children whose reaction size to 10 TU of tuberculin was 8 mm. and more. In this group the yield was 3.9 per 1,000.

On the basis of the results shown in table 3, it does not seem to be worth while to examine household contacts less than fifteen years old, or those who are contacts of the child with a reaction of 6 mm. to 7 mm. induration.

Reaction Size

The percentage distribution of the diameter of the induration in the first year of testing is illustrated in figure 3. There is a comparative leveling off of the reaction size beyond 8 mm.

During the first year of tuberculin testing, an induration measuring 6 mm. and more was called positive. Of the 1,666 children who had "positive" reactions, 47 per cent fell into the 6 mm. to 7 mm. group. This is the group which the writers believe more probably has a nonspecific reaction than reactions due to tuberculous infection. If a reaction size of 8 mm. or more had been set as the criterion for a positive reaction in the first year of testing, 4.8 per cent (876 of 22,958) would have had positive reactions instead of 7.3 per cent (1,666 of 22,958) on the basis of the 6 mm. positive criterion.

No new cases of tuberculosis were found in examining adult family associates of

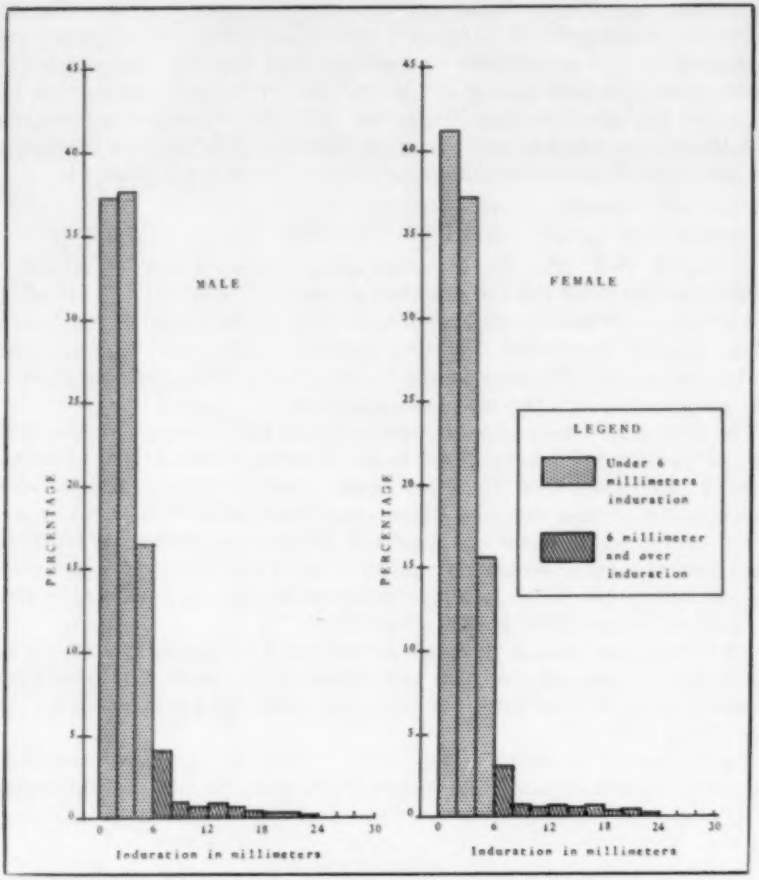


FIG. 3. Percentage distribution of reactions to tuberculin tests in the first year of tuberculin testing in selected Honolulu schools, November, 1955, to September, 1956.

children whose reaction size was 6 to 7 mm. On the other hand, it appeared that the associates of those with reactions of 8 mm. and more had a high prevalence of significant tuberculosis (table 3).

Because of the apparent evidence of the presence of nonspecific reactions, the criterion for a positive reaction has been changed upward from 6 to 8 mm., beginning in the third year of the study. In so doing, some of the children whose 6 to 7 mm. reactions do represent true infection will be overlooked. On the other hand, to consider as reactors all those with 6 to 7 mm. of induration would subject a substantial number of false positive reactors and their family associates to unnecessary examination and would increase immeasurably the cost of the project.

TABLE 4

DISTRIBUTION OF 18,394 CHILDREN ACCORDING TO THEIR REACTIONS TO THE TUBERCULIN TEST IN THE FIRST AND SECOND YEAR OF TESTING IN SELECTED HONOLULU SCHOOLS

November, 1955–August, 1957

Reaction of first year test—size of induration in mm	0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26+	
	Reaction to second year test—size of induration in mm.																										
Total Tested	6,492	2,273	3,930	2,877	1,325	411	337	156	90	43	22	56	34	33	39	23	52	35	24	30	22	19	17	12	9	33	18,394
26+															1					1						2	4
25																		2							1		3
24																		1									1
23																	2						1		1	1	5
22														1			3		1	2	1		1		1	1	11
21								1	1						1		2	1	1	2	2	3			1	1	16
20												1		1	1	1		2	2	5	1	1	1	2			5	23
19											1		2				1		1				1	2	1	3	12
18												1	1	1		3	2	4	3	3	1	1	3		1		24
17												2	2	1	4	2	3	2	1	3	2	3	2	2	1	4	34
16										1		3		4	3	3	2	2	1	4	4	4	2			3	36
15								1		3		1	2	1	10		3	3	5	1	3	2	2	2		5	44
14				1					2			2		1		1	6	3	1	3	2	2	1	2		2	29
13						1	1			6		7	3	3	2	2	5	2	2	1	3	1				2	41
12	1			2		1	1	2	3	3		2	4	10	4	3	8	8	2	1	2			1	1		59
11		1	1				3	3	3	2	4	4		1	3		4	2		1			2		1		35
10			1	1	3	2	2	6	2	2	1	1	5	2	3	3	3	2	1	2		1	1	1		1	46
9	1		2	3	3	3	7	2	14	2	1	6	1	3	1		4		1								54
8	4	2	1	5	4	4	7	12	6	4	1	8	3	1	1	2	2									1	68
7	11	5	9	8	1	16	38	14	9	5	1	7	1	1	2	2				1		1					132
6	49	11	49	68	44	83	54	29	16	5	7			2	1				2								420
5	166	67	173	174	169	44	59	27	5	1		5	2			1	1										894
4	552	225	478	422	191	56	51	16	10	3	3	1	4		1												2,013
3	1,530	566	1,074	728	302	88	34	17	7	4	3	2	1		1		1				1					2	4,361
2	984	399	615	441	176	33	20	5	2	1		2															2,678
0	3,194	997	1,527	1,024	432	80	60	21	10	1		1	3					1									7,351

Conversion and Reversion in Tuberculin Testing

In this project the plan was to test each child each year regardless of his reaction to the test the previous year. In this way it was hoped not only to identify the converters but also to gain some knowledge of the variability to be expected.

The frequency distribution of the size of the reactions between the first and second tests performed about one year apart is shown in table 4. A study of this table shows the extent of variation between the first and second years of testing. It points out the difficulty in determining just who should be considered a converter. It would appear that those with reactions of less than 6 mm. in the preceding year and 8 mm. or more in the succeeding year might be safely classed as converters, and that there is a borderline reaction size (6 to 7 mm.) which would most frequently represent a nonspecific reaction but which might include an unknown number of true tuberculin reactions.

The data given in table 5 on "conversion" and "reversion" of the tuberculin reaction between the first and second years of the study are presented only to provide further evidence that, in a mass testing program, at least, and with the dosage used in this project, a reaction should be greater than 7 mm. in size of induration before it should be called "positive." Of the 17,297 children (tested in both years) who had no reactions or reactions producing an induration of 5 mm. or less during the first year of testing, 691 had reactions of 6 mm. or more in the second year, a "conversion" rate of 4.0 per cent in one year. Conversely, of 1,100 who had reactions of 6 mm. or more in the first year, 291 were negative or had reactions of less than 6 mm. in the second year, a "reversion" rate of 26.4 per cent. On the other hand, of the 17,849 children (tested in both years) who had no

TABLE 5

"CONVERTERS" AND "REVERTERS" WITH RESPECT TO THE TUBERCULIN REACTION NUMBER AND PERCENTAGE OF CHILDREN WHOSE TESTS WERE NEGATIVE ON THE FIRST-YEAR TEST AND POSITIVE ON THE SECOND-YEAR TEST ONE YEAR LATER: ONE YEAR OF OBSERVATION

Selected Honolulu Schools, November, 1955–August, 1957

School Year	6 Mm. as Positive Criterion	8 Mm. as Positive Criterion
Conversion, 1955–1956		
Number of negatives on the first-year test retested	17,297	17,849
Number of negatives converting to positive on the second-year test	691	272
Per cent conversion	4.0	1.5
Reversion, 1955–1956		
Number of positives on the first-year test retested	1,100	550
Number of positives reverting to negative on the second-year test	291	68
Per cent reversion	26.4	12.4

reactions or whose reactions were less than 8 mm. in the first year, 272 had reactions of 8 mm. or more in the second year, a "conversion" rate of 1.5 per cent. Conversely, 68 of 550 children (tested in both years) with reactions of 8 mm. and more in the first year reverted to less than 8 mm. in the second year, a "reversion" rate of 12.4 per cent (table 5).

Discussion

Since the study was designed for a five-year period, only the obviously important findings and some of the impressions to date have been presented.

One thing that was impressive was the relative inexactness of the tuberculin reaction as a tool. By means of the tuberculin reaction it is not possible to separate the population clearly and distinctly into those who are infected with tubercle bacilli and those who are not. There was no sharp dividing line between the tuberculin reactors and nonreactors. The reactions of a large group of persons fell in a borderline group. There is strong evidence that most of the smaller reactions represent nonspecific reactions (6–8). Therefore, many, if not most, of those in this borderline group should not be considered infected.

Aside from the difficulties which arise in interpreting the test because of this "nonspecific" reaction, there are the factors of injecting an exact amount of tuberculin at the correct depth, and the actual determination of the presence (or absence) of induration and of its size. Not only is difficulty experienced in actually deciding whether induration exists, but there is no exact technique to determine within a millimeter the border of such induration if it does exist.

The size of induration varies greatly. Some reactions are well-circumscribed elevations and easy to measure. Others are soft, and the borders are extremely difficult to palpate. This latter problem seems to be particularly prevalent in the younger children. No attempt is made to read 1-mm. indurations. Erythema is recorded whenever present, and a small amount is present even in many who have no palpable induration.

Nevertheless, in spite of these difficulties in administering and interpreting the test, the writers are confident that the study is being performed in as uniform and accurate a way as possible. There has been very little turnover of the very competent staff which performs the actual testing, and purified protein derivative tablets from the same manufacturer, prepared in solution in the same way, have been used throughout these two years of study.

Summary

A five-year tuberculin-testing project of a portion of the school population in the city of Honolulu is briefly described and the results of the first two years of testing are summarized.

Approximately 7.3 per cent of the 23,000 children tested reacted to 10 TU of PPD with an induration of 6 mm. or more, and 4.8 per cent with an induration of 8 mm. and more in the first year of the project.

Two thousand three hundred and thirty-eight adult household associates, fifteen years of age or older, of school children with reactions of 6 to 7 mm. in one

or more tests were examined without finding a single new case of active tuberculosis. Neither were any cases found among the 732 adult household associates of the children whose reactions reverted from positive (6 mm. or more) on the first test to negative (less than 6 mm.) on the second test. The yield of new active cases of tuberculosis in the 2,330 adult household associates, fifteen years of age and older, of school children with reactions of 8 mm. and more on one or more tests was 3.9 per 1,000.

The yield from tuberculin testing 28,544 school children from kindergarten through high school was 8 new active cases, or 0.3 per 1,000.

The observed tuberculin conversion rate among 17,297 school children, negative in the first year of testing and re-tested approximately a year later, was 4.0 per cent per year, using 6 mm. and more as the positive criterion. The conversion rate was 1.5 per cent, using 8 mm. as the positive criterion.

The rate of "reversion" among school children from positive in the first-year test to negative in the second-year test was 26.4 per cent, using 6 mm. as the positive criterion. The reversion rate was 12.4 per cent, using 8 mm. as the positive criterion.

With the test dose of 10 TU and the technique employed in this study, it would appear that the lower limit of induration which should be called positive in a mass tuberculin-testing project in this area is 8 mm.

Sumario

Estudio Explorador de Cinco Años de Comprobación con Tuberculina en las Escuelas de Honolulu

Descríbese aquí sucintamente un plan quinquenal de comprobación con tuberculina de una porción de la población escolar de la ciudad de Honolulu, sumarizándose los resultados de los dos primeros años de comprobación.

Aproximadamente un 7.3 or ciento de los 23,000 niños comprobados reaccionó a 10 UT de PPD (DPP) con una induración de 6 mm. o más y un 4.8 por ciento con una induración de 8 mm. y más en el primer año del estudio.

Dos mil trescientos treinta y ocho convivientes adultos, de quince años de edad o más, de los escolares que acusaron reacciones de 6 a 7 mm. en una o más pruebas fueron examinados sin descubrir un solo caso nuevo de tuberculosis activa. Tampoco se descubrieron casos entre los 732 convivientes adultos de los niños cuyas reacciones viraron de positivas (6 mm. o más) en la primera prueba a negativas (menos de 6 mm.) en la segunda prueba. El rendimiento de nuevos casos activos de tuberculosis en los 2,330 convivientes adultos, de quince o más años, de los escolares que acusaron reacciones de 8 mm. o más en una o más pruebas fué de 3.9 por mil.

El rendimiento de la comprobación con tuberculina en 28,544 escolares desde las escuelas de párvulos a las escuelas superiores consistió en 8 casos activos nuevos, o sea 0.3 por 1,000.

El coeficiente de viraje de la prueba a la tuberculina observado entre 17,297 escolares, negativos en el primer año de comprobación y recomprobados aproximadamente un año después, fué de 4.0 por ciento al año, tomando 6 mm. o más como pauta de positividad. El coeficiente de viraje representó 1.5 por ciento cuando se usó el tamaño de 8 mm. como pauta positiva.

Con la dosis comprobadora de 10 UT y la técnica empleada en este estudio, parece que el límite inferior de induración que cabría llamar positivo en una obra colectiva de comprobación con tuberculina en esta zona es de 8 mm.

Resume

Cinq années d'étude-pilote du test à la tuberculine dans les écoles d'Honolulu

Un programme de cinq ans concernant le test à la tuberculine dans une partie de la population écolière de la ville d'Honolulu est brièvement décrit et les deux premières années de ce test sont résumées.

Environ 7,3% des 23.000 enfants testés ont réagi à 10 UT de PPD par une induration de 6 mm. ou plus et 4,8% par une induration de 8 mm. ou plus, au cours de la première année du programme.

On a examiné deux mille trois cent trente-huit adultes (âgés de quinze ans et au-dessus) associés au foyer d'écoliers ayant des réactions atteignant 6 à 7 mm. à l'occasion d'un seul ou de plusieurs tests, sans découvrir un seul nouveau cas de tuberculose en activité. Il n'a également été décelé aucun cas parmi les 732 adultes associés au foyer des enfants dont les réactions sont passées de positives (6 mm. ou plus) au premier test à négatives (moins de 6 mm.) au deuxième test. Les nouveaux cas de tuberculose en activité, dépistés parmi les 2,330 adultes (âgés de 15 ans et au-dessus), qui étaient associés au foyer d'écoliers ayant des réactions de 8 mm. ou plus à l'occasion d'un ou de plusieurs tests, atteignaient 3,9 pour 1.000.

Les tests à la tuberculine effectués chez 28.544 écoliers, du jardin d'enfants à "high school" inclusivement (c. à d. comprenant les écoliers âgés de seize ans environ) ont permis de découvrir 8 nouveaux cas en activité, soit 0,3 pour 1.000.

Le taux de virage observé dans le test à la tuberculine parmi 17.297 écoliers, à réaction négative lors de la première année du test et testés à nouveau approximativement un an plus tard, était de 4% par an, l'induration de 6 mm. et plus étant considérée comme le critère positif. Le taux de virage était de 1,5% lorsque l'induration de 8 mm. était considérée comme le critère positif.

Le taux de "reversion" parmi les écoliers, dont le test positif dans la première année devenait négatif lors du test de deuxiéme année, était de 26,4%, l'induration de 6 mm. étant prise comme critére positif. Le taux de reversion était de 12,4%, lorsque l'induration de 8 mm. était prise comme critère positif.

Etant données la dose du test de 10 UT et la technique employée dans l'étude, il semblerait que 8 mm. représente la limite inférieure pour une induration pouvant être qualifiée "positive" dans un programme de dépistage en masse par l'épreuve de la tuberculine dans cette région.

REFERENCES

(1) Palmer, C. E., Krohn, E. F., Manos, N. E., and Edwards, L. B.: Tuberculin sensitivity of young adults, Pub. Health Rep., 1956, *71*, 633.

(2) Yuan, I-C., and Nyboe, J.: Mass BCG vaccination in Syria, 1950, and Mass BCG vaccination in Egypt, 1949–1952, International Tuberculosis Campaign, Copenhagen, Denmark, pp. 11–12; p. 14.

(3) Bureau of Health Statistics, Department of Health, Territory of Hawaii.

(4) U. S. Public Health Service Tuberculosis Reports.

(5) Annual Reports of the Department of Health, Territory of Hawaii.

(6) Yuan, I-C., and Nyboe, J.: Mass BCG vaccination in Czechoslovakia 1948–1949, The International Tuberculosis Campaign, Copenhagen, Denmark, p. 15.

(7) Palmer, C. E., Ferebee, S. H., and Petersen, O. S.: Studies of pulmonary findings and antigen sensitivity among student nurses: VI. Geographic differences in sensitivity to tuberculin as evidence of non-specific allergy, Pub. Health Rep., 1950, *65*, 1111.

(8) Palmer, C. E.: Tuberculin sensitivity and contact with tuberculosis: Further evidence of nonspecific sensitivity, Am. Rev. Tuberc., 1953, *68*, 678.

CHEMICAL ANALYSIS OF THE ROLE OF HUMORAL FACTORS IN NATIVE AND ACQUIRED RESISTANCE TO TUBERCULOSIS[1]

SHUNSAKU OSHIMA, YUTAKA FUJITA, ATSUKO TAKEOKA, MICHIRO NAKAJIMA, AND SHUSUKE TSUJI

(Received for publication June 23, 1958)

INTRODUCTION

It has been demonstrated in previous investigations (1, 2) that the low molecular fraction (capable of passing through a cellophane membrane) of the body fluid of normal rabbits possesses a certain degree of bacteriostatic activity against virulent tubercle bacilli *in vivo* and that the high molecular fraction (incapable of passing through a cellophane membrane) inhibits this action of the low fraction and thus acts to promote growth. Therefore, the total body fluid of normal rabbits is unable to inhibit growth of virulent tubercle bacilli *in vivo*. It is of interest that avian tubercle bacilli, nonpathogenic Mycobacteria (such as *M.* 607), and *M. smegmatis* are resistant to the action of the low molecular fraction of body fluid, being capable rather of utilizing the low molecular fraction for their growth *in vivo*.

It was also demonstrated that the growth of virulent tubercle bacilli *in vivo* was completely inhibited in the body fluid of sufficiently immunized rabbits. It was inferred from the results obtained by special *in vitro* experiments (using the ring method (3)) that this increased tuberculostatic power of immune body fluid or serum was mainly attributable to the reduction in the growth-stimulating action of the protein fraction. It was postulated that a change in the albumin fraction of body fluid might play an important role in the acquired resistance of immunized body fluid to virulent tubercle bacilli.

Subsequent investigations dealing with further fractionation of both low and high molecular fractions of normal and immune body fluid will be reported in this paper. Growth experiments *in vitro* of tubercle bacilli in media containing these subfractions confirm the data obtained in the previous investigations (1–3).

MATERIALS AND METHODS

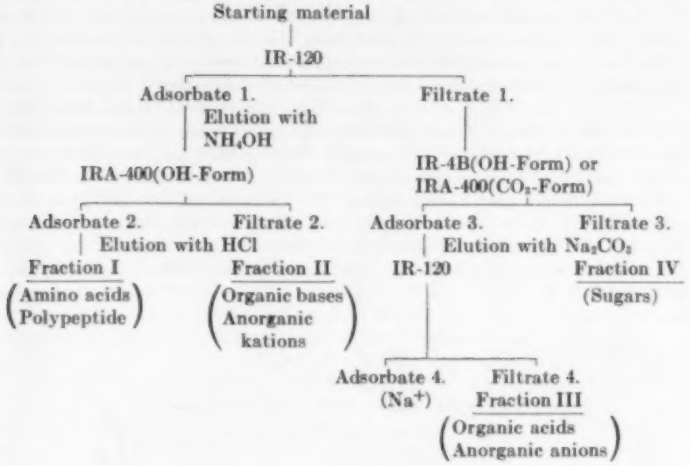
As preliminary materials of low molecular fraction of body fluid, healthy human urine (of tuberculin-positive persons), dialyzed outer fluid of normal rabbit's serum, and dialyzed outer fluid of organ extract from the muscles, livers, kidneys, and lungs of normal rabbits were prepared as follows:

Fresh serum (approximately 30 ml.) was dialyzed through a cellophane bag against a tenfold volume (300 ml.) of distilled water at 4°C. for seventy-two hours. The outer fluid was employed as a preliminary material for serum. Muscle pieces collected from freshly sacrificed rabbits were homogenated after being thoroughly washed with water. The organ homogenate was placed in a cellophane bag and dialyzed against ten- to

[1] From the Fifth Division of the Tuberculosis Research Institute, Kyoto University, Kyoto City, Japan.

TABLE 1

PROCEDURES USED IN CHEMICAL FRACTIONATION OF LOW MOLECULAR SUBSTANCES



fifteen-fold volumes of distilled water at 4°C. for seventy-two hours. The outer fluid was employed as a starting material for the organ. Fresh urine was used without dialysis as a starting material.

Technique of chemical fractionation of low molecular substances: The adsorption technique was used, using ion exchange resins. Urine must previously be treated with charcoal powder (100 ml. of urine plus 0.5 gm. of charcoal) in order to free it from colloidal substances so that it can be smoothly employed in the adsorption procedure. Although, as first observed, active charcoal is capable of adsorbing tuberculostatic substances in urine, the loss of active substances is negligible under these conditions. Procedures are summarized in table 1.

Amberlite® IR-120, H-form (strong kation exchange resin), Amberlite IRA-400, OH-form and CO_3-form (strong anion exchange resin), and Amberlite IR-4B, OH-form (weak anion exchange resin) were employed consecutively.

The resin IR-120 was warmed with approximately five times its volume of 2N HCl-solution at 80°C. for thirty minutes, and was crammed into a glass tube as described below after being washed with water. This resin column was thoroughly washed with water until the effluent solution became neutral and was used for the adsorption. For repeated use, the resin in a glass tube was reactivated with an appropriate volume of 2N HCl solution and washed.

The IRA-400 was activated with 2N NaOH solution or 1N Na_2CO_3 solution, and washed with water. The IR-4B was activated with 1N NaOH solution and was used after being washed with water. A glass tube 2.0 cm. in diameter and 50 cm. in height was used for the column. Resins were crammed in up to the 40-cm. height of this tube.

The starting material was first treated through the IR-120 column and washed with water (dropping speed: 10 to 20 ml. per minute). The effluent solution was "Filtrate 1." The resin column, after being washed thoroughly, was eluted with 2N-NH_4OH solution and washed with water until the reaction became neutral. This fluid was boiled under reduced pressure in order to remove NH_3 gas. This is "Adsorbate 1." "Adsorbate 1" was treated with IRA-400 (OH-form). The filtrate obtained was fraction II containing organic bases and anorganic kations. (In this procedure loss of a fairly large quantity of anorganic kations may be unavoidable because of the use of a weak rather than a strong alkali.)

Elution with 2N HCl solution from the IRA-400 column gave fraction I containing amino acids and polypeptides. "Filtrate 1" was treated with IR-4B or IRA-400-CO_3 form and the effluent solution obtained was fraction IV containing sugars. The eluted material with the Na_2CO_3 solution was treated again with IR-120-H-form in order to remove Na^+. The effluent solution was fraction III containing organic acids and anorganic anions.

Each fraction was concentrated under reduced pressure, was transferred to a watch glass, and liophylized to dryness in a desiccator, weighed, and reserved.

Test for tuberculostatic activity: The slide culture method was used. One milliliter of serially diluted material was added to 1.0 ml. of twice concentrated Kirchner's liquid mixed with 0.3 ml. of Eiken's albumin in which a slide had been immersed smeared with the test bacteria (H37Rv strain, unless otherwise noted) treated by the benzine method (1). Cultures were continued for ten days at 37°C. The degree of growth of bacilli is designated as +++, ++, +, ±, −. (+++: colonies adhere to each other, and there are no distinct colonies; ++: very good proliferation, but there are distinct colonies; +: good proliferation (by oil immersion); ±: sometimes slight proliferation, sometimes no growth; −: no bacillary growth).

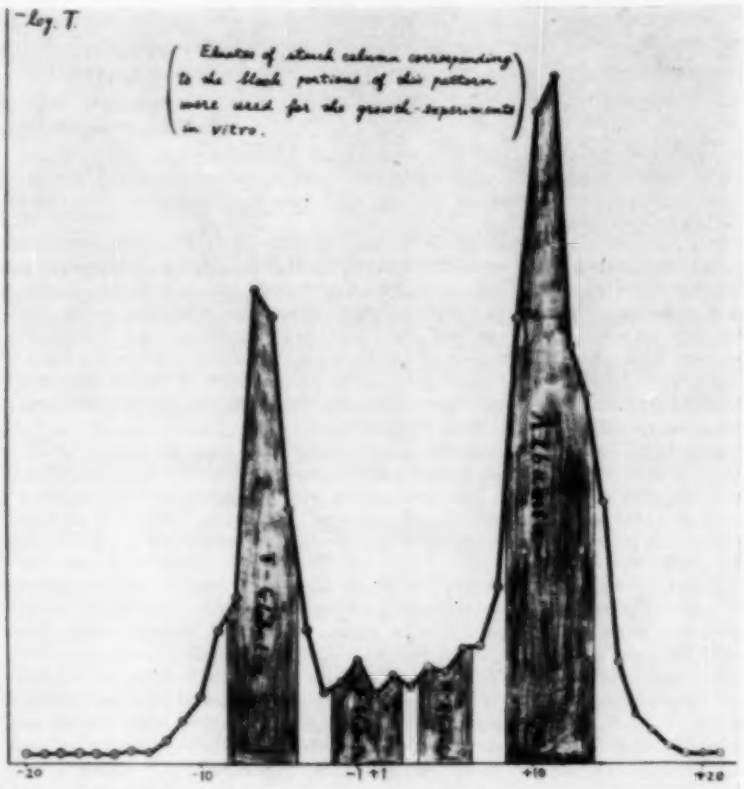


FIG. 1. Electrophoretic pattern of normal rabbit's serum plotted by using Folin-Ciocalteau's reagent.

Technique of fractionation of high molecular fraction: The zone electrophoresis technique using the starch column described by H. G. Kunkel (4) was employed for fractionation of serum protein.

Animals were bled by heart puncture or by cutting the neck artery. Serum was collected by centrifugation. Starch was previously washed with distilled water, and insoluble starch was collected in dryness. Electrophoresis of serum in the starch column was performed at 4°C. for twenty-four hours under the following conditions: veronal acetate buffer (pH 8.6, $\mu = 0.05$), 250–300 V, 2mA/cm².

When electrophoresis ended, the starch column was cut into 1 cm. long pieces. Each piece was placed in a test tube containing 10 ml. of physiologic saline solution. Elution of protein was performed with frequent stirrings at 4°C. for twenty-four to forty-eight hours.

Two-tenths of a milliliter of eluted protein solution was colored by Folin-Ciocalteau's method as follows: 200 ml. of 0.1 N NaOH solution, in which Na_2CO_3 (anhydric) had been dissolved in 2 per cent concentration, was mixed with 4 ml. 0.5 per cent $CuSO_4$ solution. Slight opalescence occurred. After thorough mixing, 3.5 ml. of this opalescent solution was poured into a tube containing 0.2 ml. of the sample and 0.4 ml. of distilled water. A blue color appeared. The colorimetric value was measured by electrophotometer. From the values obtained by colorimetry, a distribution curve of serum protein was made (figure 1).

Using this pattern, eluates corresponding to albumin, α-, β-, and γ-globulin fractions were collected in cellophane bags. Dialysis against carbowax 1500 for several hours made the protein solution appropriately concentrated. During this dialysis carbowax molecules might pass through the cellophane membrane into the solution. However, in a preliminary control experiment it has been confirmed that this concentration of carbowax had not interfered at all with the growth of the H37Rv strain *in vitro*. As determinations by the refractometer were interfered with by contaminating carbowax, the concentration of the protein solution was corrected by some other technique—in most cases Folin's reagent was used. The protein solution was diluted to a 3 to 4 per cent concentration with physiologic saline and, after being sterilized through Seitz's filter, was examined for its effect on the growth of tubercle bacilli *in vitro*.

Culture media: Using the same sample of protein solution, two different media were prepared: (*1*) 1 ml. of the sample plus 1 ml. of Kirchner's liquid without albumin (O-medium), and (*2*) 1 ml. of the sample plus 1 ml. of Kirchner's liquid plus 0.3 ml. of Eiken's bovine albumin (A-medium). O-medium was adequate for the determination of the growth-stimulating activity of the protein added, because the H37Rv strain smeared on the slide was incapable of multiplying in the deep culture using Kirchner's liquid without albumin. A-medium was used for the determination of the bacteriostatic activity of the protein added, because the medium used is complete for multiplication of the H37Rv strain.

Results

Experiments Related to the Low Molecular Substance(s)

Examination of the growth-inhibiting action using crude materials of low molecular substance: The starting materials were concentrated under reduced pressure (50 to 60 mg. of mercury, 50 to 55°C.) to $\frac{1}{100}$ volume in urine, to $\frac{1}{16}$ volume in serum, and to dryness in the organ extract. The dry substance of organ extract was dissolved in $\frac{1}{4}$ volume of the original starting material with distilled water. These concentrated materials were serially diluted and, after being sterilized at 80°C. for thirty minutes, were added to Kirchner's medium. Various strains of Mycobacteria were cultured by the slide culture method, and the growth-inhibiting action of the added materials was examined. The results are shown in table 2.

TABLE 2

GROWTH-INHIBITING ACTIVITY OF URINARY MATERIAL ON VARIOUS STRAINS OF MYCOBACTERIA

Strain	Concentration									
	64X	32X	16X	8X	4X	2X	1X*	½X	¼X	Control
H37Rv	−	−	−	−	−	+	++	++	++	++
BCG	−	−	−	−	−	+	++	++	++	++
Avian	−	+	++	++	++	++	++	++	++	++
M. 607	−	+	++	++	++	++	++	++	++	++

Slide culture in Kirchner's medium for ten days.

* 1X means the concentration of the starting material and according to the increase of number the materials are concentrated.

TABLE 3

GROWTH-INHIBITING ACTIVITY OF SERUM MATERIAL ON VARIOUS STRAINS OF MYCOBACTERIA

Low Molecular Fraction from	Strain	Concentration					
		4X	2X	1X	½X	¼X	⅛X
Bovine serum	H37Rv		−	+	++	++	++
	BCG		−	++	++	++	++
	Avian	−	−	−	++	++	++
		++	++	++	++	++	++
	M. 607	++	++	++	++	++	++
Rabbit serum	H37Rv	−	−	−	++	++	
	Avian	−	−	−	++	++	
		−	++	++	++	++	
	M. 607	++	++	++	++	++	
Dog serum	H37Rv		−	++	++	++	
	BCG		−	++	++	++	

Slide culture in Kirchner's medium for 10 days.
Control: ++.

Material from human urine inhibited growth of various virulent tubercle bacilli; however, avian tubercle bacilli and the *M.* 607 strain were hardly restricted in their growth.

In table 3 may be seen the results of serum materials of various kinds of animals. Growth-inhibiting properties similar to those in human urine were observed, except for the behavior of the avian strain. Materials from bovine and rabbit serum sometimes inhibited growth of avian bacilli.

The results of various organ materials of normal rabbits are indicated in table 4. Although great differences were not noted among antituberculous properties of various organs, it appeared that activity of the lung and the kidney was slightly less than that of the muscle, liver, and brain.

Stability: It appeared that the tuberculostatic substance(s) in the low molecular fraction were fairly heat stable, because activity remained powerful after being heated at 100°C. for sixty minutes.

Solubility: The starting material was warmed and evaporated to dryness and was immersed in various organic solvents. Soluble and insoluble portions were separated by filtration. Both portions were again warmed to dryness in order to remove solvents and were redissolved in distilled water. After sterilization at 80°C. for one hour, tuberculostatic activities were tested.

It was noted that the active substances were soluble in water and methanol and very slightly soluble in ethanol; but they were insoluble in butanol, acetone, ethyl ether, and benzene.

Activities of fractions separated by Amberlite treatment: It was to be expected that fraction I contained amino acids and polypeptides; fraction II, organic bases; fraction III, organic acids; and fraction IV, sugars. Ninhydrin and Molisch's reactions in fractions from serum materials are shown in table 5. The same tests with fractions from the muscle are shown in table 6. It appears that fractionation of the muscle material may be insufficient in its purification. It may

TABLE 4

GROWTH-INHIBITING ACTIVITY OF ORGAN MATERIAL ON VIRULENT TUBERCLE BACILLI

Organ	Concentration				
	4×	2×	1×	½×	¼×
Muscle	−	−	+	+++	+++
Liver	−	−	+	+++	+++
Kidney	−	+	++	+++	+++
Lung		+	++	+++	+++
Brain		−	+	+++	+++

Slide culture using H37Rv in Kirchner's medium for ten days.
Control: +++.

TABLE 5

QUALITATIVE EXAMINATION OF FRACTION FROM SERUM MATERIAL

	Ninhydrin	Molisch
Original material	+	+
Fraction I	+	−
Fraction II	−	−
Fraction III	−	−
Fraction IV	−	+

TABLE 6

QUALITATIVE EXAMINATION OF FRACTION FROM ORGAN MATERIAL

	Ninhydrin	Molisch
Fraction I	+	−
Fraction II	+ weak	−
Fraction III	±	−
Fraction IV	±	+

TABLE 7

GROWTH-INHIBITING ACTIVITY OF FRACTION SEPARATED FROM HEALTHY HUMAN URINE

	64X	32X	16X	8X	4X	2X	1X
Fraction I	−	−	−	++	++	++	++
Fraction II	++	++	++	++	++	++	++
Fraction III	−	−	−	++	++	++	++
Fraction IV	++	++	++	++	++	++	++

Slide culture using H37Rv in Kirchner's medium for ten days.
Control: ++.

TABLE 8

GROWTH-INHIBITING ACTIVITY OF FRACTION SEPARATED FROM MUSCLE EXTRACT OF NORMAL RABBIT

	8X	4X	2X	1X
Fraction I	+	+	+++	+++
Fraction II		+	+++	+++
Fraction III	−	−	±	+++
Fraction IV	+++	+++	+++	+++

Slide culture using H37Rv in Kirchner's medium for ten days.
Control: +++.

be inferred that more complicated substances may be contained in the muscle material than in the other materials.

The tuberculostatic properties possessed by each fraction are indicated in table 7. Fraction I from urine material sometimes showed obvious tuberculostatic activity and sometimes no activity. Fraction III demonstrated apparent activity in all experiments performed. Fractions II and IV indicated no activity. Fractions I and III from serum consistently indicated apparent activity.

Fractions of muscle material showed somewhat complicated results (table 8). It was almost determined that fractions I and III had tuberculostatic properties, although the degree of inhibiting activity was not uniform according to the starting material used. In two of the four experiments performed, fraction II showed slight inhibition of the H37Rv strain; fraction IV showed no activity.

In conclusion, it is certain that the tuberculostatic substance contained in the low molecular fraction of body fluid exists either in the form of organic acid or of amino acid or polypeptide. The significance of anorganic salts may be almost negligible because of the solubility of active substances in methanol.

Further purification and chemical identification of fractions I and III are in progress.

Experiments Related to the High Molecular Fractions

Serum protein fractions from normal animals: Human (healthy tuberculin-positive persons), bovine, rabbit, and guinea pig serum were fractionated by the zone electrophoresis technique and each fraction was tested for its effect on the

growth of tubercle bacilli. The H37Rv strain was used as the test bacterium. The results of the cultures added with the rabbit fractions diluted serially are shown in table 9. The summarized results are shown in table 10. The data are uniform in all serum materials from different animal species. The albumin fraction alone supported and promoted growth of tubercle bacilli in the O-medium. The α-, β-, and γ-globulin fractions were incapable of supporting or promoting the growth of bacilli in the O-media. In the A-media, containing albumin, α-, and β-globulin, the growth of bacilli was almost uniform and equivalent to that in the control medium. No inhibition was noted. However, in the A-medium containing γ-globulin, slight inhibition of multiplication of the H37Rv strain was noted.

From these results it may be concluded that serum albumin is the unique source of the growth-stimulating ability of serum protein, and that serum globulin has no ability to promote or support growth of virulent tubercle bacilli.

TABLE 9

INFLUENCE OF PROTEIN FRACTION SEPARATED FROM NORMAL RABBIT'S SERUM ON THE GROWTH OF VIRULENT TUBERCLE BACILLI IN VITRO

Fraction	O—Medium							A—Medium						
	1×	½×	¼×	⅛×	1/16×	1/32×	1/64×	1×	½×	¼×	⅛×	1/16×	1/32×	1/64×
Albumin	−	+	++	++	++	+	−	±	++	++	++	++	++	++
α-globulin		−	−	−	−	−	−		++	++	++	++	++	++
β-globulin		−	−	−	−	−	−		++	++	++	++	++	++
γ-globulin	−	−	−	−	−	−	−	−	+	++	++	++	++	++

Slide culture using H37Rv for ten days.

TABLE 10

INFLUENCES OF PROTEIN FRACTION SEPARATED FROM HUMAN SERUM AND SERUM OF VARIOUS ANIMALS ON THE GROWTH OF VIRULENT TUBERCLE BACILLI IN VITRO

		A—Medium	O—Medium
Human serum	Albumin	++	++
	α-globulin	++	−
	β-globulin	++	−
	γ-globulin	+	−
Bovine serum	Albumin	++	++
	α-globulin	++	−
	β-globulin	++	−
	γ-globulin	+	−
Rabbit serum	Albumin	++	++
	α-globulin	++	−
	β-globulin	++	−
	γ-globulin	+	−
Guinea pig serum	Albumin	++	++
	α-globulin	++	−
	β-globulin	++	−
	γ-globulin	+	−

TABLE 11

INFLUENCE OF PROTEIN FRACTION SEPARATED FROM SERUM OF IMMUNIZED RABBIT ON THE GROWTH OF VIRULENT TUBERCLE BACILLI IN VITRO IN COMPARISON WITH THE RESULTS OBTAINED FROM NORMAL NONIMMUNIZED RABBIT

		O—Medium					A—Medium				
		1×	½×	¼×	⅛×	1/16×	1×	½×	¼×	⅛×	1/16×
Albumin	Normal	−	++	++	++	++	++	++	++	++	++
	Immune	−	++	++	−	−	++	++	++	++	++
α-globulin	Normal	−	−	−	−	−	++	++	++	++	++
	Immune	−	−	−	−	−	++	++	++	++	++
β-globulin	Normal	−	−	−	−	−	++	++	++	++	++
	Immune	−	−	−	−	−	++	++	++	++	++
γ-globulin	Normal	−	−	−	−	−	−	+	++	++	++
	Immune	−	−	−	−	−	−	+	++	++	++

Slide culture using H37Rv for ten days.

And it appears that γ-globulin of normal serum possesses, though slightly, a growth-inhibiting power. It may be of interest, as shown in table 8, that the albumin fraction, in its very concentrated state, rather inhibited growth of tubercle bacilli.

Serum protein fractions from immunized rabbits: Rabbits were immunized with: two subcutaneous injections (at one week's interval) of 100 mg. heat-killed H37Rv strain suspended in paraffin oil, or four subcutaneous injections (also at one week's interval) of 10 mg. of the same vaccine. Four to five weeks after the last vaccination, the animals were sacrificed and their sera fractionated by zone electrophoresis.

Growth experiments, performed by adding fractions of immune sera to the A and O-media are summarized in table 11. Both immunizations had the same influence on the serum fractions. It is of considerable interest that the albumin fraction showed no appreciable growth-promoting effect in the O-medium, although normal albumin had an apparent effect as described above. On the other hand, γ-globulin slightly inhibited the growth of bacilli in the A-medium, as in the A-medium containing the γ-globulin from normal rabbits. No more powerful inhibition than that of the γ-globulin of nonimmunized serum was noted, though it might possibly be expected that increased γ-globulin of antibody might exert a powerful action on the growth of tubercle bacilli. Also α- and β-globulin fractions were unchanged in their effect on tubercle bacilli from those in normal nonimmunized states.

The same results were obtained in the two series (5 mg. and 50 mg. of living BCG vaccination) of experiments using BCG vaccine. Albumin was reduced in its growth-promoting power and γ-globulin was the same as in normal controls.

DISCUSSION

Björnesjo (5–9) conducted extensive investigations on the tuberculo-inhibitory properties of human urine and attempted to identify this substance. Also,

he (10) investigated bovine serum and various organ extracts, and he found that dialyzed fluids of these materials possessed similar or the same properties as the urine. Chemical identification was ultimately unsuccessful. However, he found various important properties of the active substances. Heat stability, solubility in organic solvents, and adsorbability by activated charcoal and ion exchange resins are in good agreement with the present investigation. Myrvik and associates (11) postulated that the properties of Björnesjo's urinary substance were not unlike those of ascorbic acid and certain of its derivatives. Björnesjo (12) criticized this information of Myrvik and associates concerning the discrepancy of solubility in methanol. The writers of the present report not only agree with Björnesjo's opinion with reference to the solubility of the active substance in methanol, but also consider that the methanol solubility is one of the most important characteristics of the active substances. However, the present writers have observed, contrary to Björnesjo's finding, that the active substances are almost insoluble in ethanol.

It was possible in the present studies to isolate substances from urine and serum in more purified form than has previously been possible. The organic acid fraction and amino acid or polypeptide fraction, isolated from the low molecular fraction of body fluid by using the adsorption technique with Amberlite, are confirmed to be tuberculostatic *in vitro*. It is inferred that these substances also have an inhibiting effect *in vivo* on the multiplication of tubercle bacilli. Moreover, it may be that these substances are the most important source of tuberculostasis of the low molecular fraction of body fluid. Investigation with reference to the organ extract (rabbit muscle extract) had a somewhat different result from those just described. Although it is certain that organic acid and amino acid or polypeptide fractions possess apparent tuberculostatic capacity, there is a possibility that other active substances exist, especially in the form of organic base.

Dubos (13–15) has suggested that there might be various substances in the tissues of normal animals which have tuberculostatic properties, i.e., fatty acids, basic amines (especially spermine and spermidine), polypeptides (so-called thymus peptide), and lysozyme. Although these or other active substances may be present in serum or body fluid and urine of normal animals, the present investigation indicates that organic acids and amino acids and polypeptides play a more important role in native resistance to tuberculosis than any other substances.

It is of great interest that Patnode and Hudgins (16, 17) reported that tissue fatty acids isolated from lungs of normal animals inhibited the growth of virulent tubercle bacilli with significant relationship to the virulences of bacilli to the hosts. Hedgcock (18) recently reported that mice fed with synthetic rations containing variable fatty acids acquired more resistance to tuberculosis than those fed with ordinary "chow" containing no fatty acids. These investigations and the present studies show that it is most likely that the fatty acid content in body fluid or tissue is rather important in the native resistance of the animal to tuberculosis.

Preliminary experiments (19) with the low molecular substances from immunized rabbits indicate that immune body fluid or serum may show behavior simi-

lar to that of the nonimmunized. Patnode and Hudgins (20), on the other hand, reported that the contents of fatty acids in the lung increased after BCG immunization in rabbits. It may be necessary to perform more detailed qualitative and quantitative examinations, using various animal species, to determine the exact role of low molecular substances in immunity to tuberculosis.

In normal rabbits it appears that the source of the growth-stimulating property of serum protein is the albumin fraction, the globulin fractions having no such effect. Dubos and Middlebrook (21) reported that, although albumin acts to promote growth, globulin has no effect. And Davis and Dubos (22, 23) found that bovine albumin fraction V was more effective in promoting growth than whole serum or crystalline bovine albumin, and that the globulin fraction had no such effect at all. Youmans and Youmans (24) conducted extensive investigations on the effect of bovine serum protein fractions on the growth of tubercle bacilli of human origin *in vitro*, and reported, contrary to Davis and Dubos, that whole bovine serum and proantithrombin best stimulated the growth of bacilli and that all other subfractions, except fibrinogen and the ultrafiltrate, also acted more or less to enhance growth.

The results reported in the present paper oppose Youmans' data, especially with regard to the effect of the globulin fraction. The globulin fraction had almost no effect on, or inhibited only slightly, the growth of a human strain of tubercle bacilli. Boissevain (25) also observed similar results.

These very complicated and contradictory results obtained by different writers may be ascribed to differences in experimental methods and materials. Many authors employed materials made by Cohn's ethanol fractionation, and it may be that protein treated with ethanol is somewhat denatured and may well act differently from its native condition. It is believed that electrophoresis is a better method than Cohn's by which to isolate protein fractions in their native forms.

It may be worthy of note that the albumin fraction, in its very concentrated state, is reduced in its growth-stimulating power when mixed with Kirchner's liquid. This fact may suggest that the albumin fraction employed was not univalent and possibly was contaminated, although in low concentration, with one or more substances having tuberculostatic properties; for example, either lipoprotein or lysozyme-like substances.

It is of interest that protein fractions isolated from serum of immunized rabbits demonstrated no more growth-inhibiting action on tubercle bacilli *in vitro* than that found in the protein fractions from nonimmune serum—only slight inhibition by γ-globulin. The main reason for the increased ability of immune serum to prevent the growth of tubercle bacilli is thought to be due to the reduction of the growth-stimulating action of the albumin fraction. These results are in good agreement with the data obtained by the previous *in vitro* experiment (by the ring method). Although the cause of the reduction of the stimulating action of the albumin fraction is unknown, it is possible that certain substances possessing growth-inhibiting ability and contaminating the albumin fraction increase after immunization. From this point of view, Myrvik and Weisser's (26) finding that lysozyme increases in the serum of guinea pigs after immunization may be significant. These results suggest that the nature of the tubercu-

lous immunity in the body fluid of rabbits may be nonspecific in view of the specific reaction of antibody.

Recently, Zitrin and Wasz-Höckert (27) reported that fraction II–III (γ- and β-globulin) and fraction IV (α- and β-globulin) isolated from tuberculous human serum by Cohn's method (10 per cent unfractioned tuberculous serum, and immune commercial globulin) inhibited the growth of tubercle bacilli inoculated into Dubos's Tween®-albumin-dextrose medium, and that the most effective fraction was fraction II–III. Serum from tuberculin-negative persons had no effect. The writers also reported that passive transfer of these effective fractions to tuberculous mice significantly elongated the median survival time of treated mice. This is rather surprising to the present writers, who confirmed previously that sera of immunized animals, even in very high concentrations, were incapable of inhibiting the growth of tubercle bacilli cultivated by the slide culture method in Kirchner's medium. Kirchner (28) found no growth-inhibiting power in tuberculous human serum in his original investigation. However, Pagel (29) indicated that most serum from patients having disseminated tuberculosis, even in almost 10 per cent concentration, inhibited the growth of tubercle bacilli, and that animal serum had no such effect. Therefore, it must be considered that human serum may behave differently from animal serum. Preliminary experiments performed by one of the writers' associates, using 10 per cent tuberculous sera, as in Zitrin and Wasz-Höckert's method, did not confirm their results. None of the sera used inhibited the growth of tubercle bacilli. Nevertheless, it is necessary to determine whether or not the differences in methods and materials employed could account for the contradictions in results.

In short, it appears very likely that increased resistance to tuberculosis in the body fluid of immunized rabbits can be attributed mainly to the reduction of albumin in its growth-stimulating activity and not to the growth-inhibiting action of increased globulin—antibody.

In may be questioned whether changes in the albumin-globulin ratio in immunized serum (albumin decrease and globulin increase), in addition to possible qualitative changes in the albumin fraction, cause immune serum or body fluids to inhibit growth of tubercle bacilli *in vitro* and *in vivo*.

Further detailed fractionation of serum protein is in progress.

Summary

Tuberculo-inhibiting activity in the low molecular fraction of human urine, serum of various animals (especially rabbits), and organ extracts of rabbits were analyzed by fractionation.

Active substances were characterized by high solubility in methanol and low solubility in other organic solvents. The consecutive absorption technique, utilizing Amberlites® gave fairly satisfactory results for all of these materials. The fraction containing organic acids and that containing amino acids and polypeptides had apparent and consistent ability to inhibit the growth of virulent tubercle bacilli *in vitro*.

The serum protein of nonimmunized and immunized rabbits was frac-

tionated by the zone electrophoresis technique, and was separated into four subfractions: albumin, α-, β-, and γ-globulin fractions; and the influence of these fractions on the growth of virulent tubercle bacilli of human origin *in vitro* was examined. The albumin fraction from nonimmunized normal rabbits stimulated growth and the γ-globulin fraction inhibited growth slightly. The α- and β-globulin fractions neither stimulated nor inhibited growth.

After immunization of the animals, the albumin fraction was apparently reduced in its growth-enhancing activity, but the globulin fraction was almost unchanged in its action on tubercle bacilli from the nonimmune state. No increased activity of the γ-globulin fraction to inhibit the growth of bacilli was noted. Therefore, it was concluded that the cause of the increased tuberculostatic activity of immune serum or body fluid which had been observed in the previous *in vivo* and *in vitro* investigations was solely ascribed to the change in albumin fraction and not to the globulin fraction.

Sumario

Análisis Químico del Papel de los Factores Humorales en la Resistencia Natural y la Adquirida a la Tuberculosis

Por medio de la fraccionación se analizó la actividad tuberculoinhibidora en la fracción molecular baja de la orina humana, en el suero de varios animales (en particular conejos) y en los extractos de órganos de conejos.

Las substancias activas se caracterizaron por solubilidad alta en el metanol y baja en otros solventes orgánicos. La técnica de adsorción consecutiva que utiliza Amberlites® dió resultados bastante satisifactorios para todas dichas substancias. La fracción que contenía ácidos orgánicos y la que contenía ácidos aminados y polipéptidos poseían capacidad evidente y constante para inhibir la proliferación de bacilos tuberculosos virulentos *in vitro*.

Las seroproteínas de conejos inmunizados y no inmunizados fueron fraccionadas por la técnica de electroforesis zonal, separándose en cuatro subfracciones: albúmina y globulinas α, β, y γ. Se estudió el influjo de estas subfracciones sobre la proliferación de los bacilos tuberculosos virulentos de procedencia humana *in vitro*. La subfracción de albúmina de conejos normales no inmunizados excitó la proliferación y la de globulina γ la inhibió ligeramente. Las subfracciones de globulina α y β no excitaron ni inhibieron la proliferación.

Después de la inmunización de los animales, se redujo aparentemente la actividad de la subfracción de albúmina para acrecentar la proliferación, pero la subfracción de globulina permaneció casi inalterada en su acción sober los bacilos tuberculosos de procedencia no inmune. No se notó aumento de la actividad de la subfracción de globulina γ para inhibir la proliferación de los bacilos. De esto se dedujo que la causa de la mayor actividad tuberculostática del inmunisuero o humor orgánico que se había observado en las anteriores investigaciones *in vivo* e *in vitro* debe imputarse exclusivamente a la fracción de albúmina y no a la de globulina.

Resume

Analyse chimique du rôle des facteurs humoraux dans la résistance naturelle ou acquise à la tuberculose

L'activité tuberculo-inhibitrice dans la fraction moléculaire basse de l'urine humaine, du sérum de divers animaux (en particulier du lapin) et les extraits d'organes de lapin a été analysée par fractionnement.

Les substances actives étaient caractérisées par leur solubilité élevée dans le méthanol et leur solubilité réduite dans d'autres solvants organiques. La technique d'adsorption

consécutive utilisant l'*Amberlites*® a donné des résultats assez satisfaisants pour chacune de ces substances. La fraction contenant les acides organiques et celle contenant les acides aminés et les polypeptides témoignaient d'une capacité constante à inhiber *in vitro* la croissance des bacilles tuberculeux virulents.

La protéine sérique des lapins non immunisés ou immunisés était fractionnée par la technique de l'électrophorèse en zone et séparée en quatre sous-fractions: albumine et fractions α-, β-, et γ-globuline. L'influence de ces fraction sur la croissance *in vitro* des bacilles tuberculeux virulents d'origine humaine a été examinée. La fraction albumine provenant de lapins normaux non immunisés stimulait la croissance et la fraction γ-globuline inhibait légèrement la croissance. Les fractions α- et β-globuline ne produisaient ni stimulation ni inhibition de la croissance.

A la suite de l'immunisation des animaux, l'activité stimulatrice de la croissance de la fraction albumine paraissait réduite, mais la fraction globuline demeurait presque inchangée quant à son action sur les bacilles tuberculeux provenant d'un état non immun. On n'a constaté aucun accroissement dans l'activité inhibitrice de la fraction γ-globuline sur la croissance des bacilles. En conséquence, la cause de l'augmentation de l'activité bactériostatique du sérum immun ou des liquides organiques, qui avait été observée *in vivo* et *in vitro* dans les investigations précédentes, doit être attribuée uniquement à la modification de la fraction albumine et non à la fraction globuline.

REFERENCES

(1) Tsuji, S., and Ito, K.: An *in vivo* method of culturing tubercle bacilli: The chamber method, Am. Rev. Tuberc., 1955, *72*, 393.

(2) Tsuji, S., Ito, K., and Oshima, S.: The role of humoral factors in native and acquired resistance to tuberculosis, Am. Rev. Tuberc., 1957, *76*, 90.

(3) Tsuji, S., Oshima, S., and Takeoka, A.: The ring method: A method for analyzing the effect of serum on the growth of tubercle bacilli *in vitro*, Am. Rev. Tuberc., 1958, *77*, 524.

(4) Kunkel, H. G.: Quoted from *Methods of Biochemical Analysis*, Vol. 1, edited by David Glick, Interscience Publishers, Inc., New York, 1954, pp. 141–170.

(5) Björnesjo, K. B.: On the effect of human urine on tubercle bacilli, Acta tuberc. scandinav., 1951, *25*, 425.

(6) Björnesjo, K. B.: On the effect of human urine on tubercle bacilli: II. The tuberculostatic effect of various urine constituents, Acta tuberc. scandinav., 1951, *25*, 447.

(7) Björnesjo, K. B.: On the effect of human urine on tubercle bacilli: III. The solubility of the tuberculostatic factor in organic solvents, and its behavior in dialysis and electrodialysis, Acta tuberc. scandinav., 1951, *25*, 457.

(8) Björnesjo, K. B.: On the effect of human urine on tubercle bacilli: IV. Some attempts to concentrate and purify the tuberculostatic factor, Acta tuberc. scandinav., 1952, *27*, 116.

(9) Björnesjo, K. B.: On the effect of human urine on tubercle bacilli: V. Experiments with the tuberculostatic factor purified from urine, Acta tuberc. scandinav., 1952, *27*, 123.

(10) Björnesjo, K. B.: On the occurrence of tuberculostatic substances in bovine organ extracts, Acta tuberc. scandinav., 1952, *27*, 134.

(11) Myrvik, Q., Weisser, R. S., Houglum, B., and Berger, L. R.: Studies on the tuberculo-inhibitory properties of ascorbic acid derivatives and their possible role in inhibition of tubercle bacilli by urine, Am. Rev. Tuberc., 1954, *69*, 406.

(12) Björnesjo, K. B.: Tuberculostatic factor in normal human urine, Am. Rev. Tuberc., 1956, *73*, 967.

(13) Dubos, R. J.: A tuberculostatic agent present in animal tissues, Am. Rev. Tuberc., 1951, *63*, 119.

(14) DUBOS, R. J.: Discussion on treatment of tuberculous meningitis and survival of bacilli in tuberculous lesions, Am. Rev. Tuberc., 1952, *65*, 637.

(15) DUBOS, R. J.: Viability of tubercle bacilli *in vivo* with and without chemotherapy, Am. Rev. Tuberc., 1953, *67*, 874.

(16) PATNODE, R. A.: Tissue fatty acids and their possible relationship to the natural resistance of rabbits to infection with human-type tubercle bacilli, Am. Rev. Tuberc., 1954, *69*, 710.

(17) PATNODE, R. A., AND HUDGINS, P. C.: Tuberculostatic effect of calf lung fatty acids on human and bovine tubercle bacilli, Am. Rev. Tuberc., 1957, *75*, 630.

(18) HEDGECOCK, L. W.: The effects of diet on the inducement of acquired resistance by viable and nonviable vaccine in experimental tuberculosis, Am. Rev. Tuberc., 1958, *77*, 93.

(19) Unpublished data.

(20) PATNODE, R. A., AND HUDGINS, P. C.: Effect of BCG vaccination on fatty acid and tributyrinase content of rabbit tissues, Am. Rev. Tuberc., 1955, *72*, 340.

(21) DUBOS, R. J., AND MIDDLEBROOK, G.: Media for tubercle bacilli, Am. Rev. Tuberc., 1947, *56*, 334.

(22) DAVIS, B. D., AND DUBOS, R. J.: The binding of fatty acids by serum albumin, a protective growth factor in bacteriological media, J. Exper. Med., 1947, *86*, 215.

(23) DAVIS, B. D., AND DUBOS, R. J.: The inhibitory effect of lipase on bacterial growth in media containing fatty acid esters, J. Bact., 1948, *55*, 11.

(24) YOUMANS, A. S., AND YOUMANS, G. P.: The effect of bovine plasma fractions on the growth of *Mycobacterium tuberculosis* var. *hominis*, J. Bact., 1950, *60*, 561.

(25) BOISSEVAIN, C. H.: Growth promotion of the tubercle bacillus by serum albumin, Proc. Soc. Exper. Biol. & Med., 1940, *44*, 110.

(26) MYRVIK, Q., AND WEISSER, R. S.: A tuberculostatic serum substance possessing lysozyme-like properties, Am. Rev. Tuberc., 1951, *64*, 669.

(27) ZITRIN, C. M., AND WASZ-HÖCKERT, O.: Preliminary experiments on passive transfer of protective humoral antibodies in tuberculosis, Am. Rev. Tuberc., 1957, *76*, 256.

(28) KIRCHNER, O.: Besitzen Tuberkulose-und Nicht-tuberkulosesera baktericides Vermögen gegenüber dem Tuberkelbazillus? Ztschr. Immunitätsforsch., 1932, *74*, 56.

(29) PAGEL, W.: The effect of blood serum on the growth of tubercle bacilli in the depth of liquid media, J. Path. & Bact., 1940, *50*, 111.

COMPARISON OF TOLERATION, ABSORPTION, AND CLINICAL EFFICACY OF SEVERAL PARA-AMINOSALICYLIC ACID PREPARATIONS IN THREE HUNDRED TUBERCULOUS PATIENTS[1, 2]

SUMNER S. COHEN, WEN Y. YUE, AND SHIH H. TSAI

(Received for publication June 23, 1958)

INTRODUCTION

Para-aminosalicylic acid (PAS) is administered in conjunction with isoniazid, streptomycin, or dihydrostreptomycin in the treatment of pulmonary or extrapulmonary tuberculosis to prevent or postpone the development of resistance to these antimicrobial agents by tubercle bacilli. In addition, Morse and his associates (1) have shown that PAS administered with isoniazid may increase the blood concentrations of active (unconjugated) isoniazid by consuming part of the active acetylating capacity of the body. Although PAS is extremely valuable in the treatment of tuberculosis, its usefulness is frequently limited because of gastrointestinal disturbances, such as nausea, vomiting, cramps, diarrhea, and anorexia associated with its administration (2–9). Various attempts have been made to decrease the gastrointestinal irritation caused by the drug. These include the spacing of dosage with regard to meals; the use of the sodium, potassium, and calcium salts as well as the ascorbic acid conjugate of PAS; administration of buffered, enteric-coated, or granular forms of the acid; and the concomitant administration of probenacid to increase the absorption of PAS in order that smaller quantities may be administered (2–7). These methods however, have not significantly decreased the incidence or severity of gastrointestinal symptoms associated with the administration of PAS (2–9). On the other hand, preliminary studies conducted recently by Small and associates (7), Shane and Krzyski (8), and Hollander and co-workers (9) have indicated that excellent gastrointestinal toleration results from the use of a para-aminosalicylate-anionic exchange resin complex. In order to further evaluate this preparation, the present study compares the toleration, absorption, and clinical efficacy of Rezipas with those of the potassium, calcium, and sodium salts of PAS.

MATERIALS AND METHODS

Rezipas®[3] is a complex consisting of an anionic exchange resin to which para-aminosalicylate is adsorbed. The complex, which contains approximately 50 per cent by weight of para-aminosalicylate, is a fine, granular material with no appreciable taste. It was administered orally in doses of 6 gm. four times daily, each dose providing 3 gm. of PAS. The potassium,[4] calcium, and sodium salts of PAS were also administered orally in doses of 3

[1] From the Medical Department, Glen Lake Sanatorium, Oak Terrace, Minnesota.

[2] This study was supported in part by research grants from The Squibb Institute for Medical Research, New Brunswick, New Jersey; and Eli Lilly and Company, Indianapolis, Indiana.

[3] The Rezipas used in this study was furnished through the courtesy of The Squibb Institute for Medical Research, New Brunswick, New Jersey.

[4] The potassium PAS used in this study was supplied through the courtesy of Eli Lilly and Company, Indianapolis, Indiana.

TABLE 1

CLINICAL BACKGROUND OF 155 PATIENTS INVOLVED IN THE STUDY OF THE CLINICAL EFFICACY OF PARA-AMINOSALICYLIC ACID PREPARATIONS

Stage of Disease	Number of Patients	Cavitation		Resections
		Single	Multiple	
Minimal	21	0	0	5
Moderately advanced	87	39	13	45
Far advanced	47	6	36	14

gm. four times daily, this regimen providing, respectively, 2.01, 2.16, and 2.64 gm. of para-aminosalicylate per dose. In most patients, isoniazid, in daily doses of 4 to 5 mg. per kg. of body weight, was administered in conjunction with the above preparations.

In the studies of toleration and absorption, 300 tuberculous patients were divided at random into four groups, each group to receive one of the four preparations. The patients were observed weekly for two to twelve months. Serum concentrations of PAS were determined in fresh blood samples taken one to four hours after the initial morning dose. The blood samples were assayed by the Marshall modification of the Bratton-Marshall test (10).

For the evaluation of clinical efficacy, 155 of the above 300 patients were selected. These selected patients had active pulmonary tuberculosis and exhibited initial sputum and gastric cultures positive for tubercle bacilli, but had not received previous hospitalization or chemotherapy. The stage of disease, extent of cavitation, and the number of previous resections in these 155 patients are indicated in table 1.

The clinical efficacy of each preparation was evaluated principally in terms of its ability to prevent or postpone the development of tubercle bacilli resistant to isoniazid or the other antimicrobial drugs employed. For this purpose, bacteriologic analyses of sputum and gastric cultures and roentgenographic examinations were performed at regular intervals during the observation period of four to twelve months.

RESULTS

Tolerance: There were no gastrointestinal disturbances in 52 of 99 patients who received the PAS-resin complex; mild symptoms occurred in 38, and moderate symptoms in 9 patients. Severe gastrointestinal disturbances were not observed in any of the patients who received this complex. Of 71 patients given sodium para-aminosalicylate, gastrointestinal symptoms were absent in 27, mild in 26, moderate in 15, and severe in 3 patients. Of 81 patients who received calcium para-aminosalicylate, gastrointestinal irritation was absent in 28, mild in 32, moderate in 16, and severe in 5 patients. There were no gastrointestinal disturbances in 8 of the 49 patients who received potassium para-aminosalicylate, mild irritation occurred in 15 patients, moderate symptoms in 24, and severe disturbances in 2 patients. These results are summarized in table 2.

Absorption: The average serum concentrations of PAS obtained with single doses of the various preparations are shown in figure 1. It may be seen that after a 6-gm. dose of PAS-resin complex, which supplied 3 gm. of PAS, maximal serum concentrations of approximately 4 mg. of PAS per 100 ml. of serum were attained within approximately one and a half hours, and remained essentially constant for the duration of the observation period. The maximal serum concentrations obtained after single 3-gm. doses of sodium and potassium para-aminosalicylates

TABLE 2

INCIDENCE AND SEVERITY OF GASTROINTESTINAL SYMPTOMS
(300 Patients)

Preparation	Number of Patients	Per Cent	Symptoms Absent	Per Cent	Mild Symptoms	Per Cent	Moderate Symptoms	Per Cent	Severe Symptoms	Per Cent
PAS-resin complex	99	100	52	52.5	38	38.4	9	9.1	0	0
Potassium PAS	49	100	8	16.3	15	30.6	24	49.0	2	4.1
Calcium PAS	81	100	28	34.6	32	39.5	16	19.7	5	6.2
Sodium PAS	71	100	27	38.1	26	36.6	15	21.1	3	4.2

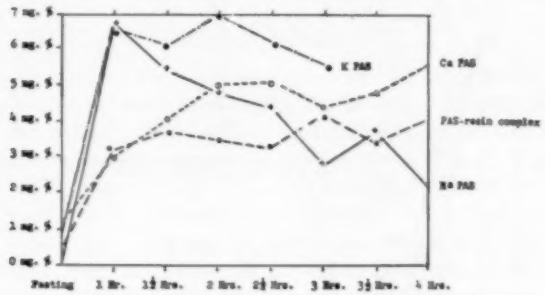


FIG. 1. Serum concentrations of acid PAS with various PAS salts.

Ca PAS—calcium PAS
Na PAS—sodium PAS
K PAS—potassium PAS

were approximately 7 mg. per 100 ml. These peak concentrations were attained within one hour and were maintained by the potassium preparation, but fell off quite rapidly in the case of sodium para-aminosalicylate. With calcium para-aminosalicylate, peak concentrations of more than 5 mg. per 100 ml. were attained slowly, and remained quite constant. It is to be noted that the absorption of these preparations varied widely from day to day, even in the same patient, as shown in table 3.

Clinical efficacy: The results of bacteriologic analyses of sputum and gastric cultures indicated that all of the forms of PAS employed were almost uniformly successful in preventing or postponing the development of resistance by the tubercle bacilli to isoniazid or to the other antimicrobial agents administered. This is shown in figure 2, in which a steady decline of gastric and sputum cultures positive for tubercle bacilli is apparent. The 21 patients with minimal disease all became noninfectious by the end of the second month of therapy and remained so. Only one of 75 patients with moderately advanced tuberculosis observed at six months of treatment had sputum or gastric culture positive for tubercle bacilli, and all patients with moderately advanced disease showed negative cultures by the tenth month of treatment. About 15 per cent of the patients with far advanced disease had cultures positive for tubercle bacilli after the

TABLE 3

RANGE OF PARA-AMINOSALICYLIC ACID SERUM CONCENTRATIONS IN MILLIGRAMS PER 100 MILLILITERS

Number of Tests	PAS-Resin Complex 158			Calcium PAS 184			Sodium PAS 207			Potassium PAS 247		
	Upper	Lower	Average	Upper	Lower	Average	Upper	Lower	Average	Upper	Lower	Average
Fasting	0.6	0.2	0.50	3.9	0.0	1.04	1.0	0.0	0.26	3.0	0.0	0.52
1 hour	5.8	1.0	3.10	8.2	1.4	4.02	10.8	4.0	6.78	12.0	1.8	6.76
1½ hours	8.4	0.8	3.73	9.2	1.0	4.07	9.3	3.0	5.44	11.2	1.2	6.20
2 hours	9.1	1.2	3.55	9.8	2.2	5.07	8.4	2.0	4.85	11.3	2.4	7.00
2½ hours	7.2	1.8	3.30	9.0	2.2	5.13	6.4	3.2	4.48	9.2	3.6	6.26
3 hours	8.4	1.4	4.22	7.6	1.6	4.46	6.0	0.8	2.76	8.8	2.2	5.60
3½ hours	5.6	1.0	3.45	4.8	4.8	4.80	5.2	1.0	3.80	—	—	—
4 hours	5.6	1.2	4.01	9.0	3.5	5.65	7.2	1.0	2.13	—	—	—

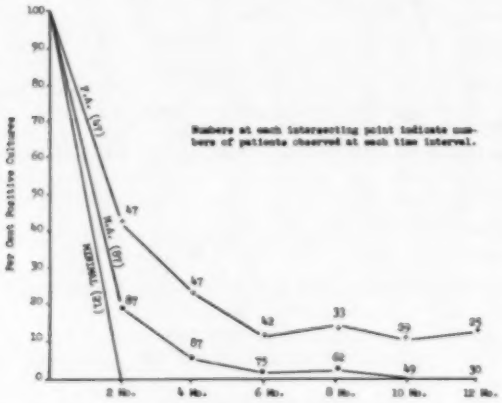


FIG. 2. Bacteriologic results.

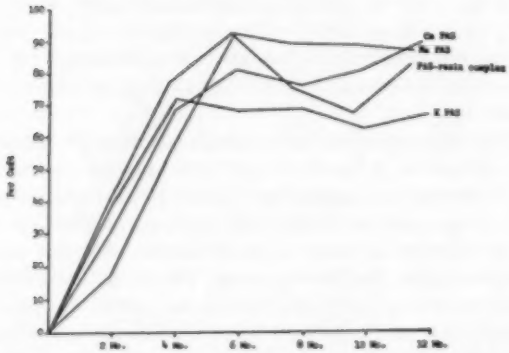


FIG. 3. Percentage of patients showing moderate to marked roentgenographic improvement at two-month intervals (after six months, too few patients remained for proper evaluation).

Ca PAS—calcium PAS
Na PAS—sodium PAS
K PAS—potassium PAS

sixth month of treatment. These satisfactory results could be attributed only in small part to the effect of resection, since the great majority of patients had cultures negative for tubercle bacilli prior to resection, which is performed at this hospital usually between the fifth and eighth months of treatment.

Patients exhibiting moderate to marked improvement, as determined by roentgenographic examination, were classified with respect to the form of PAS administered, as shown in figure 3. The patients who had undergone resection were excluded, as shown in the figure. There were no significant differences between the preparations with respect to roentgenographic improvement at six months of treatment, and too few patients remained after this time to permit comparison.

Discussion

The frequency and severity of gastrointestinal irritation associated with the administration of PAS were considerably reduced by the use of a PAS-resin complex. In this respect, the PAS-resin complex was clearly superior to potassium, sodium, and calcium para-aminosalicylate, confirming the preliminary observations of other investigators (7–9). The potassium salt, in the dosage used, supplied the least amount of para-aminosalicylate, but caused gastrointestinal disturbances in 83.7 per cent of the patients. A single dose of PAS-resin complex, on the other hand, supplied the largest amount of para-aminosalicylate, yet produced the fewest symptoms and did not cause severe symptoms in any patient. Calcium and sodium para-aminosalicylates were intermediate in this respect, producing symptoms in 65.4 and 61.9 per cent of the patients, respectively.

The rate and degree of absorption were apparently greatest with potassium para-aminosalicylate. There was no correlation between the absorption and the para-aminosalicylate content of each preparation, since the potassium salt contains the least amount of para-aminosalicylate of all preparations used. In addition, there was no suggestion that critical serum concentrations of PAS greater than those attained by any of the four preparations were necessary to provide the desired clinical effect.

Summary

Three hundred patients with minimal, moderately advanced, or far advanced pulmonary tuberculosis were divided at random into four groups, each group receiving one of the following preparations: a PAS-ion exchange resin complex, sodium para-aminosalicylate, potassium para-aminosalicylate, or calcium para-aminosalicylate. Isoniazid and other antimicrobial agents were administered in conjunction with these preparations.

Comparison of the gastrointestinal toleration of these preparations administered daily for two to twelve months indicated that a PAS-resin complex was the best-tolerated preparation, causing fewer and less severe symptoms than did the other preparations.

Studies on the rate and degree of absorption following comparable single doses indicated that no correlation exists between the absorption and the para-amino-

salicylate content of the preparations, since the potassium salt, which was most extensively absorbed, supplied the least amount of para-aminosalicylate per dose.

In 155 selected patients observed for four to twelve months, it was found that all four preparations were almost uniformly effective in preventing or postponing the development of resistance to isoniazid or the other antimicrobial agents employed. No significant differences were observed between the preparations with respect to the numbers of patients showing roentgenographic improvement.

Sumario

Comparación de la Tolerancia, la Absorción y la Eficacia Clínica de Varias Preparaciones del Acido Para-Aminosalicílico en 300 Tuberculosos

Trescientos enfermos que tenían tuberculosis mínima, moderadamente avanzada o muy avanzada fueron divididos al azar en cuatro grupos, recibiendo cada grupo una de las siguientes preparaciones: Rezipas®, para-aminosalicilato sódico, para-aminozalicilato potásico o para-aminosalicilato cálcico. Junto con ellas se administraron isoniacida y otros medicamentos antimicrobianos.

La comparación de la tolerancia gastrointestinal para dichas preparaciones administradas diariamente durante dos a doce meses indicó que el Rezipas era la mejor tolerada, provocando menos síntomas y éstos menos graves que las otras preparaciones.

Los estudios de la velocidad y la intensidad de la absorción consecutiva a dosis únicas comparables indicaron que no existe correlación entre la absorción y el contenido de para-aminosalicilato en las preparaciones, dado que la sal potásica, que fué la más absorbida, suministraba la menor cantidad de para-aminosalicilato por dosis.

En 155 enfermos escogidos, observados de cuatro a doce meses, se descubrió que las cuatro preparaciones eran casi igualmente eficaces para impedir o demorar la formación de resistencia a la isoniacida o a los otros compuestos antimicrobianos empleados. No se notaron mayores diferencias entre las preparaciones con respecto al número de enfermos que mostraba mejoría radiográfica.

Resume

Comparaison de la tolérance, de l'absorption et de l'efficacité clinique de diverses préparations d'acide para-aminosalicylique chez 300 tuberculeux

Trois cents patients atteints de tuberculose pulmonaire minimale, modérément avancée ou très avancée ont été répartis au hasard en quatre groupes, chaque groupe recevant une des préparations suivantes: Rezipas, para-aminosalicylate de sodium, para-aminosalicylate de potassium, ou para-aminosalicylate de calcium. L'isoniazide ou d'autres agents antibactériens étaient administrés en même temps que ces préparations.

La comparaison de la tolérance gastro-intestinale pour ces préparations, administrées chaque jour pendant deux à douze mois, indiquait que le Rezipas était la préparation la mieux tolérée et qu'elle produisait des réactions plus rares et moins sévères que les autres.

Les recherches sur la vitesse et le degré de l'absorption, à la suite de l'administration de doses uniques comparables, indiquaient qu'il n'existait aucune corrélation entre l'absorption et la teneur en para-aminosalicylate de ces préparations, puisque le sel de potassium, dont l'absorption était plus élevée, fournissait la plus faible quantité de para-aminosalicylate par dose.

Chez 155 patients sélectionnés, dont l'observation a duré de quatre à douze mois, on a observé l'efficacité presque uniforme des quatre préparations pour prévenir ou retarder le développement de la résistance à l'isoniazide ou autres agents antibactériens employés. Aucune différence significative n'a été observée entre les préparations à l'égard du nombre de patients présentant une amélioration radiologique.

REFERENCES

(1) Morse, W. C., Curry, F. J., Morse, P. D., Chambers, J. S., and Lincoln, A. F.: Tr. Fifteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 283.

(2) Donnerburg, R. L., Browning, R. H., and Atwell, R. J.: Studies of conjugated PAS and ascorbic acid and other forms of para-aminosalicylic acid: I. Patient tolerance, Am. Rev. Tuberc., 1957, *76*, 877.

(3) Madigan, D. G.: Para-aminosalicylic acid in tuberculosis, Lancet, 1950, *1*, 239.

(4) Boger, W. P., and Pitts, F. W.: An evaluation of certain dosage forms of para-aminosalicylic acid (PAS), Am. Rev. Tuberc., 1950, *62*, 610.

(5) Boger, W. P., Gallagher, M. E., and Pitts, F. W.: Effect of a new benzoic acid derivative on penicillin and *p*-aminosalicylic acid levels, J. Philadelphia Gen. Hosp., 1950, *1*, 51.

(6) Molthan, L., Cohen, R. V., and Zarafonetis, C.: Clinical use of potassium para-aminosalicylate (KPAS), Am. Rev. Tuberc., 1955, *71*, 220.

(7) Small, M. J., Kirman, D., and Allen, R., Jr.: Serum concentration studies with sustained-action para-aminosalicylic acid tablets, Am. Rev. Tuberc., 1958, *77*, 184.

(8) Shane, S. J., and Krzyski, T. K.: The use of a para-aminosalicylic acid-resin combination in the treatment of tuberculosis, Canad. M.A.J., 1955, *72*, 137.

(9) Hollander, A. G.: Para-aminosalicylic acid-resin complex: Studies in absorption, serum electrolytes, and tolerance, Am. Rev. Tuberc., 1955, *72*, 548.

(10) Marshall, E. K., Jr.: Determination of para-aminosalicylic acid in blood, Proc. Soc. Exper. Biol. & Med., 1948, *68*, 471.

Case Reports

PULMONARY ALVEOLAR PROTEINOSIS[1]

A Progressive, Diffuse, Fatal Pulmonary Disease

COYT R. PAYSEUR, BENJAMIN E. KONWALER, AND LEROY HYDE

(Received for publication June 23, 1958)

Introduction

A new, or heretofore unrecognized, condition which has been labeled "pulmonary alveolar proteinosis" has been reported recently. The following case history of a patient with fatal, progressive, diffuse, bilateral pulmonary disease, undiagnosed during life, is being presented in some detail as an example of this striking entity.

Case Report

A 44-year-old white, married, male clerical worker was admitted to the Long Beach Veterans Administration Hospital for the first time on March 9, 1953. Six months prior to entry he had noted the onset of dyspnea, low-grade fever, weight loss, and cough productive of approximately 0.5 ounce of whitish mucoid sputum daily. Roentgenographic studies of the chest by his personal physician, Dr. Reginald Smart of Los Angeles, in February, 1953, revealed infiltration in the right upper lobe with a cavity at the level of the right first costal cartilage. There were also extensive nodular, peribronchial infiltrations at both lung bases extending from the lower lung roots to the diaphragm. Two sputum cultures were heavily positive for tubercle bacilli. Treatment with 1 gm. of streptomycin twice weekly, intramuscularly, and 12 gm. of para-aminosalicylic acid (PAS) daily by mouth was started on February 19, 1953, prior to hospitalization.

The past history of the patient revealed that he had had pneumonia in 1943, with no known residuals. He smoked approximately one and one-half packs of cigarettes daily.

Physical examination on entry to the Long Beach Veterans Administration Hospital revealed an alert white male weighing 152 pounds. The systolic blood pressure was 142 mm. of mercury, and the diastolic pressure was 110 mm. The pulse rate was 108 per minute. Cavernous breath sounds were heard over the apex of the right lung, but no rales were heard. There was no evidence of clubbing of the fingers. The remainder of the physical examination was normal. The chest roentgenogram of March 18, 1953 (figure 1) revealed bilateral lower lobe and right upper lobe infiltrates. Planigrams revealed a cavity in the right upper lobe. Routine blood counts and urinalyses were normal. The sedimentation rate was 36 mm. in one hour, which is slightly but definitely elevated. Several sputum cultures were positive for tubercle bacilli.

Streptomycin and PAS were continued and 300 mg. of isoniazid daily by mouth were added. The patient became afebrile and asymptomatic. Numerous sputum and gastric cultures were negative for tubercle bacilli after April 21, 1953. Serial chest roentgenograms revealed progressive clearing of the lung infiltrations, and by May 8, 1953, roentgenograms revealed minimal right apical infiltration. On November 16, 1953 (figure 2), only linear fibrosis was present at the right apex. The patient was discharged on November 30, 1953, to continue his tuberculostatic drugs and to be under the care of his personal physician.

The streptomycin was discontinued in July, 1954, and PAS and isoniazid were stopped in August. The patient felt fine and resumed his work at that time. Serial roentgenograms of the chest revealed no change until April, 1956, at which time infiltrations were noted

[1] From the Pulmonary and Pathology services of the Veterans Administration Hospital, Long Beach, California, and the departments of Medicine and Pathology, U.C.L.A. School of Medicine, Los Angeles, California.

FIG. 1. Roentgenogram of March 18, 1953, at time of first hospital admission, reveals infiltration throughout most of both lungs.

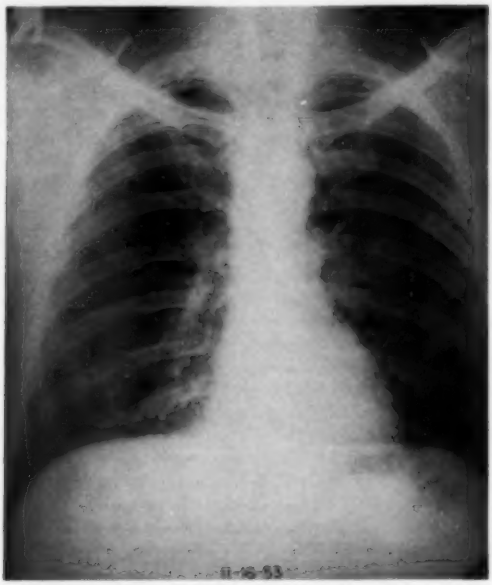


FIG. 2. Roentgenogram of November 16, 1953, after nine months of tuberculostatic chemotherapy, reveal marked clearing of exudative dsease with residual linear fibrotic strands beneath the right first anterior rib.

FIG. 3. Roentgenogram of September 25, 1956, on readmission to the hospital. All sputum and gastric cultures had been negative for tubercle bacilli for more than three years.

in both inferior perihilar areas. At that time the patient had no symptoms, and gastric washings pooled with sputum for three days were negative for *M. tuberculosis* both by culture and guinea pig inoculation. The patient was advised to re-enter the hospital, but refused at first. Chemotherapy was begun outside the hospital in April, 1956, with 1 gm. of streptomycin intramuscularly three times weekly, 300 mg. of isoniazid by mouth daily, and 6.25 gm. of PAS daily. He re-entered the Long Beach Veterans Administration Hospital on September 25, 1956, with a history of six weeks of dyspnea and a 10-pound weight loss.

Physical examination on his second hospital admission revealed a rather tremulous white male who was not acutely ill. The systolic blood pressure was 126 mm. of mercury, and the diastolic pressure was 90 mm. The pulse rate was 84 per minute and the temperature, 99.2°F. Complete physical examination revealed no abnormalities. No rales or dullness were noted upon examination of the lungs.

The chest roentgenogram of September 25, 1956 (figure 3) revealed fine nodular infiltration throughout the lower two-thirds of both lung fields. Residular fibrotic strands were noted in the right apex. Planigrams revealed no cavity. The roentgenographic appearance of the lower lung fields was quite similar to that of March, 1953. Routine blood counts, urinalyses, blood urea nitrogen, and serologic tests for syphilis were normal. The tuberculin skin test with intermediate-strength purified protein derivative was negative, but with second-strength it was positive. The coccidiodin reaction to 1:100 was negative, and the histoplasmin skin test was positive. Serologic tests for coccidiomycosis, histoplasmosis, and blastomycosis were negative. Throughout this hospitalization all of the numerous sputum specimens and gastric aspirates were negative for tubercle bacilli on culture and guinea pig inoculation.

On admission, the patient showed no evidence of cyanosis or clubbing of the fingers, and

FIG. 4. Roentgenogram of April 30, 1957, revealed increasing opacification.

FIG. 5. Roentgenogram of May 16, 1957, taken one day prior to death.

the low-grade fever disappeared after the first week. Chemotherapy was continued with streptomycin (increased to 1 gm. daily) from September 26, 1956, to November 26, 1956, when the streptomycin dosage was reduced to 1 gm. twice weekly. Isoniazid, 300 mg. daily, was begun on September 26, 1956, and was increased to 600 mg. daily on May 2, 1957. In spite of tuberculostatic chemotherapy and bed rest, serial chest roentgenograms revealed increasing pulmonary opacification (figure 4). The patient developed dyspnea on the slightest exertion and cyanosis of the nail beds.

Numerous studies were done to determine the cause of the progressive diffuse pulmonary disease. These included normal bone marrow, negative lupus erythematosus preparations of the blood, normal liver function tests, and sputum specimens negative for tumor cells. A supraclavicular node biopsy revealed "lymphoid hyperplasia." Arterial oxygen saturation on March 22, 1957, was 44 per cent. After ten minutes of 100 per cent oxygen inhalation this rose to 91 per cent. The hemoglobin rose steadily during a period of several months to 17.8 gm. per 100 ml., and the hematocrit rose to 57 per cent. The patient became more dyspneic and cyanotic at rest. He was digitalized and given intermittent oxygen therapy, tetracycline, Amesec®, and Isuprel® by nebulizer, with little, if any, benefit.

On February 7, 1957, prednisolone was begun in a dosage of 60 mg. daily. The dosage was increased to 80 mg. daily on April 15, 1957, and was continued for the remainder of the patient's hospital course. No improvement was noted subjectively or on the chest roentgenogram. On May 16, 1957, the patient complained of severe right-sided chest pain, and shortly thereafter his blood pressure was unobtainable. The temperature was 104°F. rectally. Dullness to percussion, harsh breath sounds, and rales were noted over the right lateral chest on physical examination. A portable chest roentgenogram (figure 5) showed more extensive opacification. Treatment with Levophed® and intravenous hydrocortisone resulted in restoration of normal blood pressure, but the patient became comatose and died on May 17, 1957.

Necropsy Findings

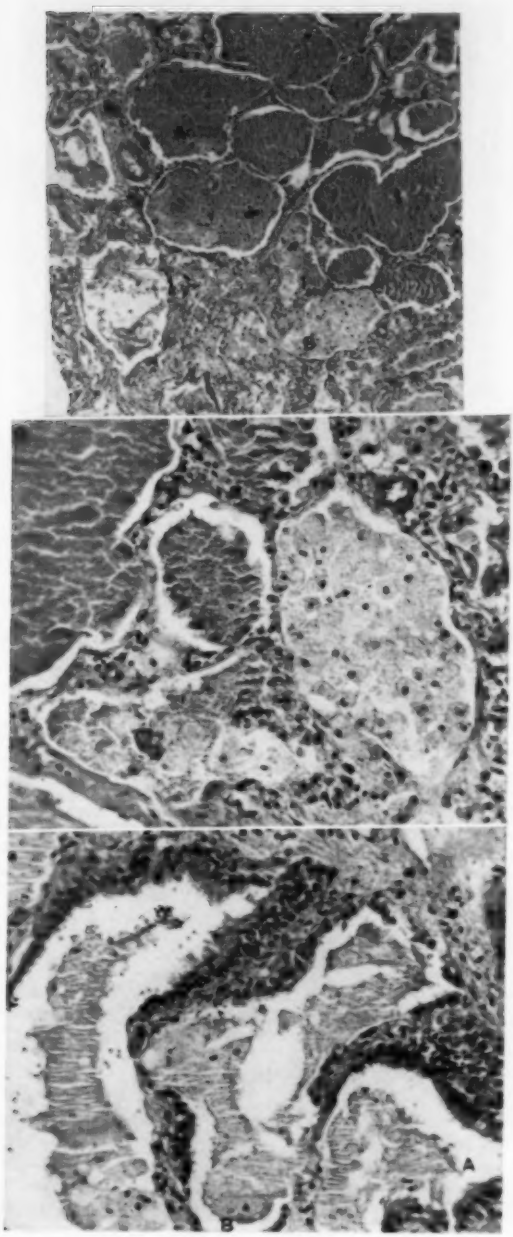
Significant findings were limited to the lungs and heart. The lungs were considerably heavier than normal; the left lung weighed 1,500 gm. and the right lung, 1,950 gm. The pleural surfaces were smooth, thin, and glistening except in the right apical area which was scarred and wrinkled. There were moderate subpleural anthracotic markings. The lung parenchyma was very firm and, even under pressure, no depressed areas were created. When blocks of pulmonary tissue were placed in water, they sank to the bottom of the container. The cut surfaces of the entire lung, both right and left, showed almost complete loss of the normal architecture with subtotal replacement by a grayish-white firm tissue. Scattered throughout this tissue were small collections of dark, anthracotic material and, occasionally, small pinkish areas of partially aerated lung tissue. There was no gross evidence of fibrosis, and no cavitation was present. The bronchial tree was patent. There was no significant lymphadenopathy involving the tracheal, tracheobronchial, or bronchopulmonary nodes.

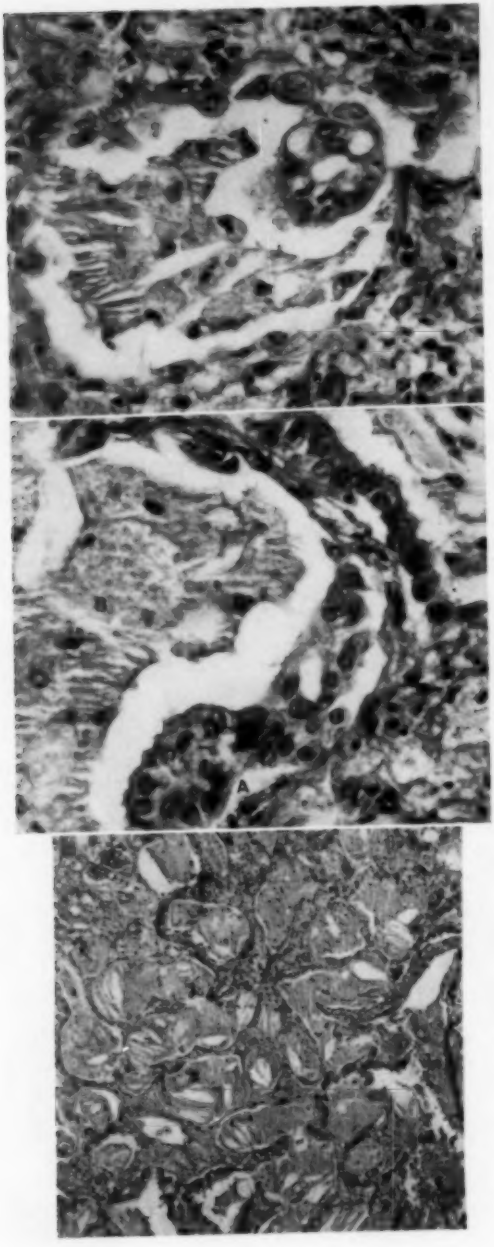
Microscopic examination of numerous blocks with hematoxylin-eosin stain revealed many alveoli either partially or solidly filled with an eosinophilic granular amorphous material. This material had distended some of the alveoli sufficiently to cause a marked

FIG. 6. (Top) Alveoli filled with amorphous material "*A*" or foamy histiocytes "*B*." Note thin septae and absence of inflammatory cells. This amorphous material is strongly positive with PAS stains and negative with muci-carmine stains. (Hematoxylin and eosin stain, × 100)

FIG. 7. (Center) High power of figure 6. Relative absence of inflammatory cells. No fungi or bacteria could be demonstrated with differential stains. (Hematoxylin and eosin stain, × 300)

FIG. 8. (Bottom) Terminal bronchioles containing amorphous material "*A*" and occasional foamy histiocytes "*B*." There is a suggestion that the granular material is arising from the breaking down of histiocytes. (Hematoxylin and eosin stain, × 300)


A
B


A

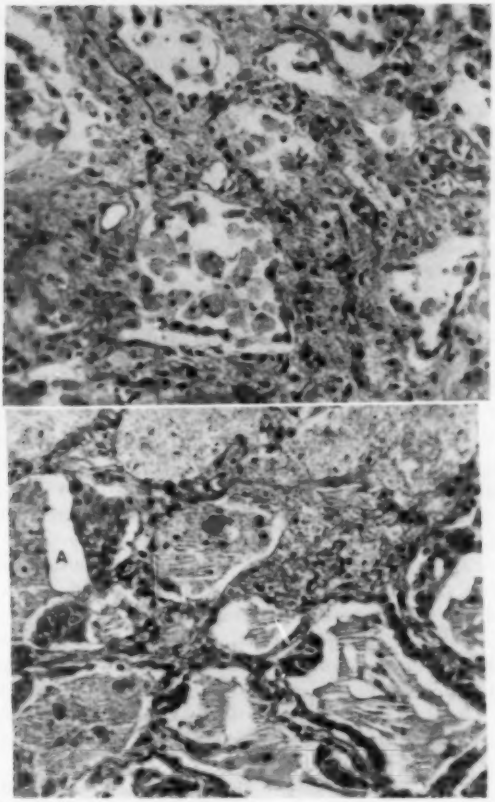


FIG. 12. (Upper) Epithelization of alveolar septae and occasional foamy histiocytes in the septae. (Hematoxylin and eosin stain, × 300)

FIG. 13. (Lower) Epithelization with formation of occasional squamatoid plaque "*A*" and formation of cellular fragments with some lamination within the cytoplasm of the foamy septal cells "*B*." These simulate inclusions. (Hematoxylin and eosin stain, × 300)

thinning or even rupture of the septae (figures 6 and 7). Occasional small bronchioles contained collections of similar material while other bronchioles contained almost colorless mucoid material (figure 8). Some alveoli were partially to completely filled with swollen foam cells that arose from proliferated septal cells (figure 9). Occasionally, the proliferated

FIG. 9. (Top) Septal cells proliferating into lumen and forming foamy histiocytes. Note degeneration of some of the foamy cells, with cleftlike spaces in some areas. These may be artifacts. (Hematoxylin and eosin stain, × 715)

FIG. 10. (Center) Note proliferated septal cells forming squamatoid areas at "*A*" and foamy histiocytes at "*B*." The latter tend to rupture. (Hematoxylin and eosin stain, × 715)

FIG. 11. (Bottom) Note numerous cleftlike spaces in the amorphous material. Oil red-O stains showed small collections of lipid material in most alveoli with occasional larger collections. (Hematoxylin and eosin stain, × 100)

septal cells formed small sheets of cells with squamatoid features (figure 10). In some areas, the swollen, proliferated septal cells were undergoing degeneration, possibly forming some of the granular material seen within the alveoli. Other alveoli contained degenerated material in which cleftlike spaces resembling cholesterol or lipid crystals were seen (figure 11). There were areas of epithelization and occasional foam cells in the interstitial tissue (figures 12 and 13). Inflammatory cells were conspicuously absent and no granulomas were seen. In some areas, acidophilic fragments were noted within the cytoplasm. These simulated inclusions (figure 13, "B"). With PAS stains, the intra-alveolar material was strongly positive. With Oil red-O stains, a minimal amount of lipid was seen in most of the alveoli, but some contained more prominent collections of lipid. The intra-alveolar material failed to stain with muci-carmine stains. The differential stains would indicate that the material was probably a muceprotein and not a lipoprotein.

The heart weighed 375 gm. The right ventricle measured 5 to 6 mm. in thickness, indicative of cor pulmonale. No other abnormalities were noted grossly or microscopically.

Numerous special stains for bacteria, parasites, and fungi were negative.

Discussion

The report on pulmonary alveolar proteinosis (1) appeared as this case report was being submitted for publication. The present case is referred to as "Case Number 9" in that paper. However, several points must be clarified. It is inferred (1) that this patient did not have tuberculosis, since none was found at necropsy in 1957. On his admission in 1953, the diagnosis was either acute hematogenous miliary tuberculosis or pulmonary tuberculosis with massive bilateral bronchogenic dissemination—more likely the latter. His first two sputum concentrates in March, 1953, were *heavily positive* for acid-fast bacilli, and cultures yielded confluent growth of tubercle bacilli. Two additional sputum concentrates and cultures in April, 1953, were positive for tubercle bacilli and there appears to be no doubt that this patient had extensive active, far advanced pulmonary tuberculosis in 1953. As noted above, his disease responded well to chemotherapy and his chest roentgenograms cleared almost completely (*See* figure 2). (The additional statement in the above-mentioned article, that pulmonary lesions persisted for several years (1), is therefore not quite correct. Lesions reappeared on chest roentgenograms in April, 1956, and these increased steadily (*See* figures 3–6)).

Pulmonary alveolar proteinosis, a rare disease, produces the clinical picture of Hamman-Rich syndrome with increasing shortness of breath accompanied by cough, fatigue, chest pain, weight loss, and cyanosis. Physical examination of the lungs was noncontributory and the patient was afebrile. The diagnosis can be made only at autopsy. The suggestion has been made that this disease may be related to inhaled pulmonary irritants, but much further study is necessary.

Summary

The present study is a report of a rare, progressive, diffuse, fatal pulmonary disease. This entity has recently been given the name "pulmonary alveolar proteinosis." The clinical findings in the case suggested the Hamman-Rich syndrome, but no pulmonary fibrosis was found at necropsy. Instead, the alveoli were filled with eosinophilic material which apparently was a mucoprotein and not a lipoprotein, although free lipid was also found.

Sumario

Proteinosis Neumoalveolar: Enfermedad pulmonar, Evolutiva, Difusa, Letal

El estudio actual versa sobre una afección pulmonar, rara, evolutiva, difusa, letal. Esta entidad he recibido hace poco el nombre de "proteinosis neumoalveolar" (1). Los hallazgos

clínicos en el caso apuntaban al síndrome de Hamman-Rich, pero no se descubrió fibrosis pulmonar en la autopsia. En vez de ello, los alvéolos estaban llenos de substancia eosinófila que aparentemente era una mucoproteína y no lipoproteína, aunque también se encontraron lípidos libres.

Resumé

Protéinose alvéolaire du poumon: Une affection pulmonaire progressive, diffuse et fatale

Cette étude constitue un rapport sur une affection pulmonaire rare évolutive, diffuse et fatale. Cette entité a été récemment dénommée "protéinose alvéolaire pulmonaire" (1). Les signes cliniques observés dans ce cas suggéraient le diagnostic de syndrome de Hamman-Rich, mais la sclérose pulmonaire n'a pas été décelée à l'autopsie. Par ailleurs les alvéoles étaient remplies d'une substance éosinophile ayant l'apparence d'une mucoprotéine et non d'une lipoprotéine, bien qu'on ait décelé également un lipide non combiné.

REFERENCE

(1) Rosen, S. H., Castleman, B., and Liebow, A. A.: Pulmonary alveolar proteinosis, New England J. Med., 1958, *258*, 1123.

PAPILLOMA OF THE BRONCHUS[1]

S. SOCHOCKY

(Received for publication August 1, 1958)

Introduction

While a papilloma of the larynx or of the trachea is not uncommon, a benign papilloma of the bronchus is one of the rarest tumors. Various writers have reported cases of papilloma of the bronchus *either* associated with a papilloma of the larynx or trachea *or* occurring in the bronchus after removal of a papilloma tumor from the larynx or trachea.

In 1929, Holding (1) described a case of multiple papillomas of the larynx, of the trachea, and of the bronchus. In 1932, Jackson (2) reported a case of multiple papillomas of the trachea with a similar lesion in the right main bronchus. In 1931, Zamora (3) removed papillomas from the larynges of patients who later developed similar lesions in the bronchi; and, in 1950, Buffmire, Clagett, and McDonald (4) described papillomas of the larynx, trachea, *and* of the right main bronchus.

Two cases of papilloma of the bronchus, both causing bronchial obstruction with atelectasis and bronchiectasis, are described below.

Benign Papilloma

Case 1. C. T., aged 45, was admitted to this hospital in October, 1956, with a history of a small hemoptysis. Family history did not reveal anything abnormal; the patient had never been seriously ill. At the time of admission the patient complained of a periodic cough for years, with a moderate amount of sputum; the cough was usually worse in winter.

A few weeks prior to admission to this hospital the patient had a spell of coughing which lasted longer than usual and his sputum was streaked with blood. He had no dyspnea on exertion. His appetite was good; weight was normal; bowel movements and micturition were normal; he smoked five to ten cigarettes daily. No abnormalities were found in either the chest or other systems on physical examination. A complete blood count revealed no abnormality; a urinalysis was normal. A chest roentgenogram showed a collapse of the posterior segment of the right lower lobe.

On bronchoscopy, the larynx, trachea, and carina appeared normal. The left side was normal, the right main bronchus, right upper lobe, and right middle lobe were normal. At the level of the right middle lobe opening, the right lower lobe stem bronchus was blocked by yellow pus which on aspiration revealed a nodular raspberry-like tumor completely occluding the lumen. A biopsy was taken. The findings were consistent with those of a carcinoma of the bronchus, which is technically operable.

Three of the biopsy fragments comprised edematous bronchial mucosa showing patchy and often focal infiltration with lymphocytes and plasma cells. The epithelium was altered in limited areas, but possibly only secondarily to these changes, i.e., points of squamous metaplasia and zones of irritative hyperplasia. The fourth fragment was mainly bronchial cartilage but also showed heavy lymphocytic infiltrations around the mucous glands.

Bronchial aspirate: Cytology (modified Dudgeon H & E): Purulent basophilic aspirate with a small proportion of eosinophils and very occasional alveolars.

A right lower lobectomy was performed a few days after admission, and the report on the specimen is as follows:

Macroscopic findings: This specimen showed a marked pleural reaction over the lateral firm zone above the diaphragmatic surface; this reaction was rectangular in outline and measured 3.5 to 4.5 cm. An incision along the bronchus disclosed an area of old basal segmental bronchiectasis. The intervening parenchyma was almost entirely destroyed and the condition was cylindrical in type. A plug of material was removed from the lining mucosa

[1] From the Thoracic Surgical Unit, Papworth, Cambridge, England.

FIG. 1. *Case 1.* Photograph of the specimen shows a papilloma in the right lower bronchus causing the cylindrical bronchiectatic changes in the basal segments of this lobe.

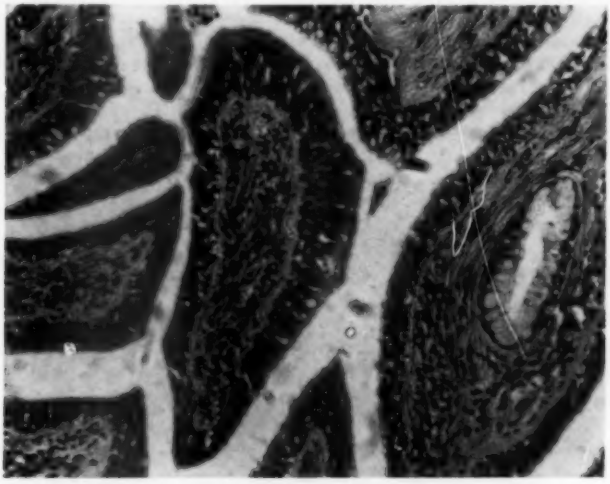


FIG. 2. *Case 1.* Photograph of the sections showing the characteristic features of the bronchial papilloma

of the main bronchus and another similar mass remained and appeared to be firmly attached to the wall of a small bronchus; this could have been organized fibrin plug. A portion of both plugs were removed for section. The apical portion of the lower lobe appeared normal; it was about half the size of the diseased area (figure 1).

Microscopic findings: The sections of the fleshy mass blocking the bronchus showed this to be a papilloma, which was covered by normal respiratory epithelium, the underlying core being infiltrated by plasma cells. When edema was marked, epithelial metaplasia was present, affecting only a small percentage of the total surface area. Sections of the ectatic segment showed massive chronic inflammatory changes with associated vascularization and ulceration of the bronchial mucosa. This chronic inflammation extended into the surrounding lung, which was the seat of quite massive fibrosis. In these areas lymphocytes were the predominant cells (figure 2).

Malignant Papilloma

Case 2. F. O., aged 41, was admitted to this hospital for pneumonectomy in September, 1954, with a diagnosis of carcinoma of the right bronchus. He was well until three months previously when he had a severe cold and a productive cough followed by tightness of the chest. On roentgenographic examination at this time, a segmental lesion was noted at the right base, which was thought possibly to be an aspiration pneumonitis, and the patient was discharged. As the roentgenographic appearance of the lesion did not improve, he was admitted to a local hospital for bronchoscopy. The report, two weeks later, was as follows:

> Right side only examined. Carina sharp and no evidence of carinal gland involvement. Main bronchus and upper lobe bronchus normal. Stem bronchus normal to beyond the apex of the lower lobe. Just beyond this point lay a fungating mass projecting from the lateral and posterior bronchial wall. An aspirate specimen was taken and biopsy from the mass, following which there was considerable bleeding. This is almost certainly a bronchial carcinoma, and bronchoscopically would appear to be operable.

The biopsy report showed a squamous cell carcinoma (figure 3).

Following this report the patient was admitted to this hospital for right pneumonectomy. On admission, his general condition was good. Apart from a slight cough with a small amount of yellow sputum, he had no symptoms attributable to the chest. Bowel movements and micturition were normal. He smoked ten to fifteen cigarettes daily. No abnormalities were found either in the chest or in other systems on physical examination. A urinalysis was normal. Chest films showed a collapse of the posterior segment of the right lower lobe. The results of a preoperative bronchoscopy were as follows: The cords, trachea, and carina were normal. The right side was first examined and the upper, middle, and apical lower

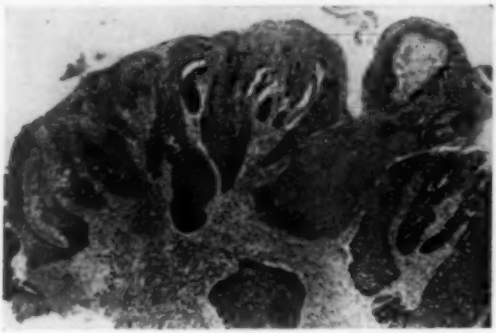


FIG. 3. *Case 2.* Photograph showing a squamous carcinoma developing in the bronchial papilloma.

FIG. 4

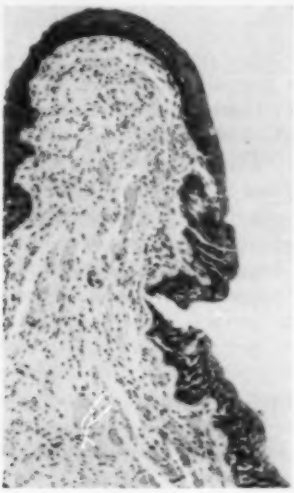


FIG. 5

FIG. 4. *Case 2.* Photographs of the specimen showing the chronic inflammatory changes distal to the papilloma.

FIG. 5. *Case 2.* Photograph of the sections showing the characteristic squamous metaplastic changes in the bronchi distal to the papilloma.

lobes were normal. Just beyond the apical lower lobe opening on the right, there was a small projection from the posterolateral wall. This had the appearance, possibly, of a tumor, but it bore no resemblance to the "ulcerating, fungating mass" that was seen at the previous examination. Possibly the removal of this by biopsy and the preoperative chemotherapy had had this effect. A right pneumonectomy was performed in the usual way.

Macroscopic findings: Palpation of the right lung and bronchial stump indicated a general firmness of the posterior and lateral segments of the lower lobe, and an incision along the bronchi disclosed bronchiectasis with chronic lung changes (figure 4). There was no obvious carcinoma but a few bronchopulmonary and pulmonary lymph nodes showed white areas which could indicate chronic inflammatory or malignant invasion. At the commencement of a posterolateral branch there was a small papillary mass, about 0.2 cm. in diameter (part of this was removed and sectioned and gave a microscopic picture similar to that of the original biopsy).

Microscopic findings: Microscopy of the right lung, bronchial stump, and hilar lymph node revealed that the ectatic bronchi had fibrosed and the walls were chronically inflamed. The basement membrane was often enormously thickened. The epithelium was altered; in the mid basals it secreted mucus and was metaplastic, atrophic, or destroyed by ulceration. In the apical lower bronchi, a sharply defined squamous metaplasia was noted (figure 5). In the main bronchus at the proximal end, there was extensive hyperplasia with metaplasia which in the distal end either is, or closely simulates, an epithelial neoplasm, e.g., some transected papillary outgrowths are indistinguishable from a bronchial carcinoma. Parenchymal changes are relative to the bronchiectasis. The pulmonary, bronchopulmonary and hilar lymph nodes were reactive.

The diagnosis was extensive metaplasia and more limited neoplasm of bronchial epithelium secondary to obstruction of the bronchi by the papilloma in the nonmalignant phase.

Summary

Two cases of papilloma of the bronchus are described, there being no evidence of this lesion in the trachea or larynx. Both papillomas were situated in the right lower lobe, producing obstruction of the bronchus and causing distal bronchiectasis and chronic nonspecific inflammatory changes.

In the first case the papilloma was benign; in the second case, however, malignant changes occurred and this papilloma was removed in toto by biopsy at the first bronchoscopy, the sections showing carcinomatous changes in the papilloma. Later macroscopic and microscopic examination of the resected lung showed evidence of very limited carcinomatous changes in the wall of the bronchi.

Both patients are symptom free and carrying on full-time jobs.

Sumario

Papiloma del Bronquio

Describense 2 casos de papiloma del bronquio, sin que hubiera signos de la lesión en la tráquea o la laringe. Ambo papilomas estaban situados en el lóbulo inferior derecho, produciendo oclusión del bronquio y bronquiectasia distal y alteraciones inflamatorias anespecíficas crónicas.

En el primer caso, el papiloma era benigno; sin embargo, en el segundo había alteraciones malignas y este papiloma fué extirpado *in toto* con la biopsia en la primera broncoscopia, revelando los cortes alteraciones carcinomatosas en el papiloma.

El subsiguiente examen macro y microscópico del pulmón resecado reveló signos de limitadísimas alteraciones carcinomatosas en la pared de los bronquios.

Ambos enfermos se encuentran asintomáticos y trabajando a tiempo completo.

Resume

Papillome de la bronche

Deux cas de papillome bronchique sont décrits, aucun signe de cette lésion n'existait dans la trachée ou le larynx. Ces deux papillomes étaient situés dans le lobe inférieur droit, ils entraînaient l'obstruction de la bronche, une bronchiectasie distale et des lésions inflammatoires chroniques non spécifiques.

Dans le premier cas, le papillome était bénin; dans le second, des signes de transformation maligne ont été décelés et l'excision chirurgicale totale du papillome par biopsie a été pratiquée au cours de la bronchoscopie initiale. Les coupes révélaient la transformation carcinomateuse du papillome.

Ultérieurement, l'examen macroscopique et histologique du tissu pulmonaire réséqué a démontré l'existence de modifications carcinomateuses très limitées dans la paroi bronchique.

Ces deux malades n'ont plus aucun symptôme et ils accomplissent un travail normal.

REFERENCES

(1) Holding, A. F.: Papillomatosis of the larynx and trachea, New York State J. Med., 1929, *29*, 271.

(2) Jackson, C., and Jackson, C. L.: Benign tumors of the trachea and bronchi, J.A.M.A., 1932, *99*, 1747.

(3) Zamora, A. M.: Two benign growths removed by bronchoscopy, J. Laryng. & Otol., 1931, *46*, 829.

(4) Buffmire, D. K., Clagett, O. T., and McDonald, J. R.: Papillomas of the larynx, trachea and bronchi, Proc. Staff Meet., Mayo Clin., 1950, *25*, 595

Notes

PRELIMINARY OBSERVATIONS ON THE DEVELOPMENT OF ATYPICAL (CHROMOGENIC) VARIANTS OF THE H37Rv STRAIN OF M. TUBERCULOSIS UNDER THE INFLUENCE OF STREPTOMYCIN AND ISONIAZID IN VITRO[1]

Interest in the significance of atypical Mycobacteria which have been implicated in pulmonary disease and other human infections has been greatly intensified in recent years. The relationship of these organisms to other Mycobacteria has been the subject of much controversy and investigation.

This study was undertaken to determine whether exposure *in vitro* of a virulent human strain of *M. tuberculosis* to any of the antituberculous agents would produce atypical (chromogenic) variants; and if so, in what ways, if any, the variants differed from the parent organism. This preliminary note concerns the development of such variants and the methods by which they were obtained. A detailed description of their morphologic, cultural, pathologic, allergenic, and antimicrobial susceptibility characteristics will be presented in a subsequent report.

Streptomycin,[2] isoniazid,[2] and the H37Rv strain of *M. tuberculosis*[3] were selected for study. Ten media were used: American Trudeau Society, Corper-Cohn, glycerol-blood agar, Löwenstein-Jensen, Tween®-albumin liquid, extract and oleic-acid-albumin agars, nutrient, brain heart infusion, and thioglycollate broth. These were prepared exactly as described previously.[4-7]

The drugs were dissolved in sterile triple-distilled water. Appropriate dilutions were always made from freshly prepared stock solutions. All culture media and reagents were checked for sterility. Smears were stained by the Gram and Ziehl-Neelsen methods.

To obtain dispersed growth, the H37Rv parent organism was grown in Tween-albumin medium[4] for seven days. At the end of this time each tube of this medium was inoculated with 0.1 ml. of the parent culture which contained approximately 1.2×10^7 viable bacterial units (as determined by the drop plate method[8]). Subsequently, 0.1 ml. inocula were used in serial passage in the Tween-albumin medium containing increasing concentrations of the antimicrobial agents which had been prepared separately for each series. Serial passage on Löwenstein-Jensen medium was made by single transfers, using 4-mm. inoculating loops.

---

[1] These studies were initiated in the Research Laboratory, Veterans Administration Hospital, Walla Walla, Washington, and were repeated and extended at the following institutions: Department of Bacteriology, University of Oregon Medical School, Portland, Oregon; Tuberculosis Research Laboratory, Veterans Administration Medical Teaching Group Hospital, Memphis, Tennessee; Department of Bacteriology, University of California, Berkeley, California; and in the present laboratory.

[2] Obtained from several different manufacturers.

[3] Fresh subcultures kindly supplied by Dr. William Steenken, Jr., Trudeau Laboratory, Saranac Lake, New York.

[4] Fisher, M. W.: Am. Rev. Tuberc., 1948, *57*, 58.

[5] Tarshis, M. S., and Frisch, A. W.: Am. Rev. Tuberc., 1952, *65*, 278.

[6] Tarshis, M. S., *et al.*: J. Bact., 1953, *66*, 448.

[7] Tarshis, M. S.: J. Bact., 1954, *67*, 117.

[8] Tarshis, M. S., and Elberg, S. S.: Am. Rev. Tuberc., 1956, *74*, 84.

Homogenates of each chromogenic variant were prepared by grinding large quantities of the organisms in sterile mortars containing from 2 to 3 ml. of a 0.1 per cent aqueous solution of crystallized bovine plasma albumin.[9] Serial dilutions of the homogenates were prepared in the same diluent to the 1.2×10^{-7} end point. Approximately 0.1 ml. aliquots from each dilution were inoculated into each of the ten types of culture media mentioned. The media were then incubated for twelve weeks at 37°C. All cultures positive for tubercle bacilli were serially subcultured twenty-four times into each of the same ten media.

In addition, 1.0-ml. amounts of the homogenates of each strain, which contained approximately 1.2×10^8 viable bacterial units, were inoculated into pairs of young male tuberculin-negative guinea pigs ranging in weight from 250 to 385 gm. Some of the surviving animals were sacrificed two weeks after being infected; others were sacrificed after four weeks. Before autopsy each animal was soaked first in 2 per cent amphyl disinfectant for five minutes, then in 70 per cent isopropyl alcohol for five minutes.

Sections of lymph node, spleen, liver, and lesions were removed aseptically with sterile instruments. Each tissue section was placed into a separate sterile mortar containing from 2 to 3 ml. of the albumin diluent and a small amount of sand. Homogenates of each tissue were also prepared. Some were serially diluted as already described; others were left undiluted. Smears and cultures were made from each tissue dilution. The ten media employed for the culture of the chromogenic variants were also used to culture the tissue homogenates. These were incubated for twelve weeks at 37°C. All cultures positive for tubercle bacilli were serially subcultured twenty-four times into each of the same ten media. Finally, 1.0-ml. quantities of each undiluted tissue homogenate were inoculated intraperitoneally into other pairs of pigs, and the serial cultures and animal passages previously mentioned were repeated twelve times each in four separate experiments. All procedures were carried out under a bacteriologic safety hood.

An initial experiment was designed to determine whether the H37Rv strain would produce atypical (chromogenic) variants when it was exposed to streptomycin in Löwenstein-Jensen medium. Tubes of media were prepared at appropriate intervals to contain the drug in the following concentrations: 0.05, 0.1, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 γ per ml. Three tubes of media were used for each concentration of streptomycin, and three tubes without the antimicrobial were used for controls.

The organism was transferred at monthly intervals to tubes of fresh media containing increasing concentrations of the antimicrobial, and to the control tubes. This procedure was continued until resistant variants could be grown at the 100.0 γ concentration.[10] Serial transfers to fresh tubes of media containing 100.0 γ of the drug per ml. were continued at monthly intervals.

After fourteen months it was seen that large numbers of yellow colonies were growing among the parent colonies in the tubes of media containing the streptomycin, but not in the control tubes. Some colonies were morphologically similar to the parent colonies. Others were greatly different, and later developed into a variety of bizarre types. On continued incubation, all of the colonies changed in color from yellow to yellow-orange, and finally to orange

Smears of the chromogenic colonies revealed numerous nonacid-fast bacilli and granules which were gram positive. Some cells resembled the parent organism while others exhibited varying degrees of pleomorphism. A number of acid-fast granules were present, some of which were observed within the nonacid-fast rods. On continued subculture at

[9] The Armour Laboratories, Chicago, Illinois.

[10] In other experiments, serial transfer of the H37Rv parent strain at twenty-four-hour intervals in Tween-albumin medium,[4] containing increasing concentrations of streptomycin and isoniazid separately (from 0.01 to 100.0 γ per ml. of medium), produced resistant, but not atypical, variants in three to four weeks at values ranging from 10.0 to 100.0 γ per ml.

monthly intervals for an additional three months on Lōwenstein-Jensen medium containing 100.0 γ of streptomycin per ml., the variant became completely nonacid-fast.

To determine whether similar findings would be obtained by using a different medium and another antituberculous agent, the experiment was repeated, using Tween-albumin liquid medium[4] instead of Lōwenstein-Jensen and isoniazid in addition to streptomycin. The procedures were the same as in the initial experiment, with two exceptions. First, the concentrations of each agent were changed to 0.1, 0.5, 1.0, 5.0, 20.0, 40.0, 50.0, and 100.0 γ per ml. of medium. Second, serial transfers of the organism, with several exceptions, were made at two-week instead of monthly intervals.

The results in this experiment were essentially the same as those in the first, except that chromogenic-resistant variants of the H37Rv strain were first observed after eighteen months instead of fourteen, as was the case in the initial experiment. On continued subculture of the variant strains, completely nonacid-fast bacilli were obtained after twenty-one months in the Tween-albumin medium containing the streptomyicn, and after twenty-two months in the medium containing the isoniazid. The streptomycin and isoniazid atypical chromogenic variant strains were designated as streptomycin-CV and isoniazid-CV, respectively.

To determine the purity of the chromogenic variants, various staining, grinding, serial dilution, subculture, animal inoculation, and re-isolation procedures were done. In no instance did the chromogens contain either mixtures of the parent organism or other mycobacterial strains.

Finally, attempts were made to ascertain whether serial passage of the variants in guinea pigs would cause reversion to the parent strain. Thus far, all such attempts have failed.

* * *

The question of whether atypical Mycobacteria are isolated more frequently from patients who have received antimicrobial therapy has been raised many times in recent years. Although such organisms had been observed often in the pre-chemotherapy era, recent observations indicate that chemotherapy may be responsible, in part at least, for the increased incidence of such strains. Some investigators[11-13] have observed that since 1945 the number of chromogenic acid-fast bacilli isolated on routine culture for tubercle bacilli has increased from 10 to 25 per cent. Bogen[14] believes that this is due to the fact that atypical Mycobacteria have only been noted more often in cultures negative than in those positive for tubercle bacilli, and that there are now more such negative cultures. This may be true. However, since it is now known that atypical acid-fast bacilli are susceptible to some of the antituberculous agents,[15-17] chemotherapy should also play a role in suppressing the growth of these organisms.

The influence of antimicrobial drugs and other agents on bacterial variation has been described by others. Graessele and Frost,[18] Bellamy and Klimek,[19] and Gale and Rodwell[20]

[11] Schiff, J., and Tarshis, M. S.: Northwest Med., 1950, *49*, 451.

[12] Quarterly Progress Report of the Veterans Administration-Armed Forces Study on the Chemotherapy of Tuberculosis, 1957, *12*, 38.

[13] Other investigators: Personal communications.

[14] Bogen, E.: Personal communication.

[15] Tarshis, M. S., *et al*: Am. Rev. Tuberc., 1955, *72*, 115.

[16] Wolinsky, E., *et al*: Am. Rev. Tuberc., 1957, *76*, 497.

[17] Rogul, M., *et al*: Am. Rev. Tuberc., 1957, *76*, 697.

[18] Graessle, O. E., and Frost, B. M.: Proc. Soc. Exper. Biol. & Med., 1946, *63*, 171.

[19] Bellamy, W. D., and Klimek, J. W.: J. Bact., 1948, *55*, 153.

[20] Gale, E. F., and Rodwell, A. W.: J. Gen. Microbiol., 1949, *3*, 127.

observed significant changes in morphology, staining, and metabolic activity for Staphylococci made resistant to penicillin and streptomycin *in vitro*. Dienes[21] found that penicillin causes the production of "L" forms of *Streptobacillus moniliformis* and the bacteroides. Voureka[22, 23] showed that profound morphologic and cultural alterations, including loss of virulence, may sometimes result in strains of *E. coli* and *P. aeruginosa* obtained from the urine of patients receiving chloramphenicol. When a strain of *E. coli* was treated with specific antiserum and chloramphenicol, alterations in morphology, sugar fermentation, resistance to inimical agents, and antigenic structure could be induced. Yegian and Budd[24, 25] observed the heterogeneous character of streptomycin-dependent mutants obtained from strains of Mycobacteria and called attention to the possible role of bacterial inheritance in variation. Marshak[26] reported the differences in response of the H37Rv and H37Ra strains of human tubercle bacilli to a number of metabolites and presented evidence of their differences by gene mutation. Karlsson[27] was able to induce metabolic mutants with strain 607 of *M. tuberculosis* by ultraviolet radiation. The effect of streptomycin and X-radiation on strains of *E. coli* and the role of genetic mechanisms in variation was also discussed by Tatum,[28] Newcombe and Hawirko,[29] and Lederberg.[30] The effect of isoniazid, cycloserine, and α-ethylthio isonicotinamide on morphology and loss of acid-fastness of the H37Rv strain of *M. tuberculosis* and other Mycobacteria was reported by Middlebrook,[31] Schaefer,[32] Koch-Weser and associates,[33] Barclay and Russe,[34] and Dunbar.[35]

Although the mechanisms which operate to produce atypical strains of tubercle bacilli and many of the basic factors favoring variation have not been clearly understood, work in recent years on bacterial inheritance and genetic aspects of bacterial resistance to antimicrobial agents indicates that many drug-resistant variants arise by spontaneous mutation. Tarshis and Frisch[36] observed that the H37Rv strain of *M. tuberculosis* grown for long periods on Corper-Cohn[37] and Löwenstein-Jensen[6] media develops chromogenic variants which exhibit atypical staining and pathogenicity. Similar observations were made by Miller.[38, 39]

What mechanisms were involved in the production of such variants is not clear. Steenken[40] pointed out that spontaneous alteration of human and bovine strains of tubercle

[21] Dienes, L.: J. Bact., 1948, *56*, 445.
[22] Voureka, A.: Lancet, 1951, *1*, 27.
[23] Voureka, A.: Lancet, 1951, *1*, 28.
[24] Yegian, D., and Budd, V.: J. Bact., 1951, *61*, 161.
[25] Yegian, D., and Budd, V.: J. Bact., 1951, *61*, 167.
[26] Marshak, A.: J. Bact., 1951, *61*, 1.
[27] Karlsson, J. L.: J. Bact., 1954, *68*, 592.
[28] Tatum, E. L.: Proc. nat. Acad. Sci. (Wash.), 1945, *31*, 215.
[29] Newcombe, H. B., and Hawirko, R.: J. Bact., 1949, *57*, 565.
[30] Lederberg, J.: J. Bact., 1951, *61*, 549.
[31] Middlebrook, G.: Am. Rev. Tubere., 1952, *65*, 765.
[32] Schaefer, W.: Am. Rev. Tubere., 1954, *69*, 125.
[33] Koch-Weser, D., *et al.*: Am. Rev. Tubere., 1955, *71*, 556.
[34] Barclay, W. R., and Russe, H.: Am. Rev. Tubere., 1955, *72*, 236.
[35] Dunbar, J. M.: Ann. Inst. Pasteur, 1957, *92*, 451.
[36] Tarshis, M. S., and Frisch, A. W.: Am. Rev. Tubere., 1952, *65*, 302.
[37] Corper, H. J., and Cohn, M. L.: Am. J. Hyg., 1933, *18*, 1.
[38] Miller, F. R.: Science, 1931, *74*, 343.
[39] Miller, F. R.: J. Exper. Med., 1932, *56*, 411.
[40] Steenken, W. Jr.: Am. Rev. Tubere., 1950, *62*, 22.

bacilli can occur after years of subculture on artificial media, and that atypical avirulent variants can be produced in a relatively short period by controlling one or more factors during subculture on special media. Typical virulent tubercle bacilli can become chromogenic if grown in a medium containing small amounts of ferric citrate,[41] or deeply colored bovine serum,[42] or if the pH is altered.[43] Such variants exhibit different degrees of alteration from their parent-strain characteristics. A similar *in vivo* transformation is suggested by the reports of Pinner,[44] Wenkle and associates,[45] and Schiff and Tarshis,[11] all of whom noted that, when chromogens were isolated from patients, many of them were in a clinically improved phase of their disease.

The last two investigators[11] also reported the isolation of a virulent, atypical, chromogenic acid-fast bacillus from the cerebrospinal fluid of a patient with pulmonary tuberculosis and tuberculous meningitis. He had been given streptomycin, the amount he received being a total of 232.2 gm. intramuscularly in 129 days and a total of 9.5 gm. intrathecally in 125 days. During treatment, typical virulent tubercle bacilli were recovered in the initial as well as in subsequent specimens of sputum and cerebrospinal fluid. The chromogen was isolated from the cerebrospinal fluid in pure culture at three different times, namely, 117, 142, and 233 days following the initiation of intrathecal therapy. In the same report it was pointed out further that 19 per cent more atypical, chromogenic Mycobacteria were isolated from patients who were treated with streptomycin than from those who did not receive such therapy. It was postulated, therefore, that the drug might possibly be interfering in some way with the metabolism of virulent Mycobacteria and that this was possibly responsible for the production of chromogenic mutants.

Some of the intrinsic physico-chemical mechanisms which operate not only in altering the course of microbial infection and disease, but also in modifying the characteristics of the organisms, were discussed in detail by Dubos.[46]

The numerous studies reported during the last thirty years[5, 36, 40, 47] demonstrate that dissociation can be accomplished with various Mycobacteria and that a wide variety of atypical strains can be produced. In addition, all grades of pathogenicity are possible from single strains, depending on the environmental and other factors which can be varied either experimentally or by chance. Stability of the variant strains and reversion to the parent type have also been the subject of much controversy.

Of special interest in the studies herein reported is the fact that, despite the frequent and numerous subcultures of the chromogens to a variety of drug-free media and their serial passage in guinea pigs, the variants have thus far not reverted to the parent type. However, this does not preclude the possibility that reversion could be achieved under a different set of conditions. For example, serial passage of the chromogenic variants for twelve weeks in American Trudeau Society and Tween-albumin media containing an extract of the parent H37Rv strain produced some substrains which were similar to the parent organism. By similar methods, Miller[39] was able to cause reversion of nonacid-

[41] Reed, G. B., and Rice, C. E.: J. Bact., 1929, *17*, 407.

[42] Griffith, A. S.: Lancet, 1916, *1*, 721.

[43] Steenken, W. Jr.: J. Infect. Dis., 1935, *56*, 273.

[44] Pinner, M.: Pulmonary Tuberculosis in the Adult, ed. 1, Charles C Thomas, Springfield, Illinois, 1946.

[45] Wenkle, W. C., *et al.*: Am. Rev. Tuberc., 1948, *57*, 385.

[46] Dubos, R. J.: Biochemical Determinants of Microbial Diseases, ed. 1, Harvard University Press, Cambridge, Massachusetts, 1954.

[47] Tarshis, M. S., and Frisch, A. W.: Am. Rev. Tuberc., 1952, *65*, 289.

fast bacilli of the H37 strain of *M. tuberculosis* to the acid-fast form from which they were derived. Chromogenic variants of virulent human tubercle bacilli could be made to revert to their parent types by serial animal passage.[38, 48]

Steenken[48] pointed out that such methods are open to question. Since none of the cultures were initiated by single-cell isolates of the parent organism, there may have been a few parent cells present which developed when the variant strains were inoculated into animals or when subcultured. Such a possibility exists. However, all staining, grinding, serial dilution, subculture, animal inoculation, and reisolation procedures used to determine the purity of the cultures indicated that the organisms used in the present investigations were not mixed. Single-cell studies would be worth while in order to obtain additional evidence for such reversion phenomena.

The question of whether contaminating organisms may have entered the cultures during the course of these investigations must be seriously considered. However, in experiments performed at different times, in different laboratories, and in different parts of the country, similar results were obtained. Thus, if the atypical variants were contaminants, it would have to be postulated that fresh stock cultures in different localities were being contaminated with the same organisms. In addition, the variants appeared in the drug-containing media, but not in the control media. Some colonies, at least, should have appeared in the latter as well, unless, of course, the drug-free controls were not conducive to their growth.

In summary: Investigations were undertaken to determine whether exposure *in vitro* of the H37Rv strain of *M. tuberculosis* to any of the antituberculous agents would produce atypical (chromogenic) variants and, if so, in what ways (if any) the variants differed from the parent organism.

Streptomycin- and isoniazid-resistant, but not atypical, variants were observed in three to four weeks after serial passage of the parent organism every twenty-four hours in Tween-albumin liquid medium containing increasing concentrations of each agent separately. By continued serial passage of the parent strain, first at monthly, then at two-week intervals, resistant, atypical variants were seen after fourteen months of exposure to 100.0 γ of streptomycin per ml. in Löwenstein-Jensen medium, and after eighteen months' exposure to the same concentration of both drugs separately in Tween-albumin medium. No atypical variants were observed in the control media without these agents.

By the procedures used, under the conditions described, it is concluded that streptomycin- and isoniazid-resistant, atypical, nonacid-fast variant strains can be produced. These organisms have been designated as streptomycin-CV and isoniazid-CV, respectively. Additional studies are in progress to elucidate further the development of variant strains of Mycobacteria under the influence of antimicrobial agents.

MEDICAL RESEARCH LABORATORY
VETERANS ADMINISTRATION HOSPITAL
ALEXANDRIA, LOUISIANA

MAURICE S. TARSHIS

January 1, 1958

[48] PINNER, M.: Am. Rev. Tubere., 1935, *32*, 440.

CYCLOSERINE COMBINED WITH PYRAZINAMIDE IN THE TREATMENT OF PULMONARY TUBERCULOSIS

One of the major problems in the treatment of pulmonary tuberculosis is the management of the patient whose organisms are already resistant to isoniazid, streptomycin, and para-aminosalicylic acid (PAS), and whose sputum remains positive for acid-fast bacilli.

The effectiveness of cycloserine compared with other antituberculous drugs is not well established. When cycloserine is used alone, studies have suggested that its action is less potent than either streptomycin or isoniazid.[1, 2] McLean[3] and Livings[4] state that cycloserine is not the drug of choice for initial treatment.

Cycloserine is absorbed readily through gastric mucosa after ingestion and its excretion into the urine is rather prompt.[5] Therefore there is no accumulation phenomenon. The toxic effects of cycloserine are manifested primarily in the central nervous system[6] and generally develop during the first months of treatment. The severity and frequency of the toxic reactions are related to the total daily dose and the frequency of administration. Many investigators[7, 8] agree that the toxicity of cycloserine can be minimized by giving less than 1.0 gm. daily and by keeping the blood concentration of cycloserine under 30 γ per ml. There is a difference of opinion regarding the value of analeptics in preventing the epileptiform seizures.[9, 10] Nair[11] states that pyridoxine had a definite effect on tremor and hyperreflexia.

The emergence of resistance to cycloserine is similar to that seen with isoniazid or streptomycin.[12, 13] Storey[14] noted that some organisms were resistant to 15 γ of cycloserine per ml. prior to treatment.

The purpose of the present study was to investigate the usefulness of cycloserine and

[1] Schmidt, L. H.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 353.

[2] Cuckler, A. C., *et al.*: Antibiotics & Chemother., 1955, *4*, 191.

[3] McLean, R. L.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 387.

[4] Livings, D. G.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 372.

[5] Welch, M., *et al.*: Antibiotic Med., 1955, *1*, 72.

[6] Epstein, I. G., *et al.*: Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 326.

[7] Epstein, I. G., *et al.*: Antibiotics Annual, 1955–1956, p. 141.

[8] Murray, F. J.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 332.

[9] Barclay, W. R., *et al.*: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957, p. 57.

[10] Ravina, A., *et al.*: Antibiotics Annual, 1955–1956, p. 148.

[11] Nair, K. G. S., *et al.*: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957, p. 54.

[12] Cummings, M. M.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 377.

[13] Moyer, R. E., and Schwartz, W. S.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 380.

[14] Storey, P. B.: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957, p. 44.

pyrazinamide in patients whose organisms are resistant to streptomycin, isoniazid, and PAS.

Twenty-five patients were selected for the study. All were long-term, chronic patients with sputum consistently positive for tubercle bacilli. Organisms from these patients were consistently resistant to streptomycin (100 γ per ml.), isoniazid (5 γ per ml.), and PAS (100 γ per ml.). All patients received cycloserine[15] (250 mg. every twelve hours) with pyrazinamide (1 gm. three times daily). These drugs were given for a minimum of six months unless complications necessitated change.

Roentgenograms of the chest and sputum or gastric cultures were obtained monthly. Drug-susceptibility studies were done for isoniazid, PAS, streptomycin, and cycloserine prior to drug therapy and on all tubercle bacilli recovered thereafter. Cycloserine-susceptibility tests were performed by inoculating Löwenstein-Jensen medium containing 10, 20, and 80 γ of cycloserine per ml.

Twenty patients completed the full course of therapy. Five patients failed to complete the six months of study: two died from massive hemoptysis during treatment, two died from pulmonary insufficiency, one had therapy interrupted because of severe urinary tract infection.

In cases in which the sputum became negative for tubercle bacilli, the drugs were continued as specific therapy, assuming the calculated risk of drug toxicity.

Cycloserine concentrations of the blood were determined after one and three months of treatment, using Johnes' method.[16] Blood samples were obtained before the morning (8 a.m.) dose of cycloserine, at 2 p.m., and before the evening (8 p.m.) dose of cycloserine.

There were no serious toxic reactions in the 20 patients treated with cycloserine-pyrazinamide, and they tolerated the treatment well. The mild reactions are presented in table 1.

Headache and drowsiness were the main side effects with this therapy. These symptoms occurred within nine days after the start of medication and lasted three to five days, after which the symptoms disappeared spontaneously.

There was one patient whose medication had to be discontinued for ten days because of nausea and anorexia; otherwise, the patients continued treatment without any complications. Two patients experienced upper and lower extremity joint pain which occurred one month after the start of treatment. There was no evidence of swelling or redness of the joints, but there was some limitation of motion. The joint pains disappeared without discontinuation of the drugs.

None of the patients developed convulsions or hyperreflexia, and none developed liver damage from pyrazinamide. The group is not large enough to evaluate the existence of buffer phenomenon between cycloserine and pyrazinamide regarding toxic effects.

There was no significant difference in cycloserine concentrations in the blood after the first and third months of therapy. The individual mean cycloserine concentrations are presented in table 2.

The average cycloserine concentration in the blood before the morning dose was 15.1 γ per ml. and the range was 5.8 to 30.6 γ per ml. Before the evening dose it was 14.3 γ per ml., with a range of 5.1 to 31.9 γ per ml. There was no significant fluctuation between the morning and evening concentrations but, six hours after the morning dose, the cycloserine concentration in the blood was slightly higher, with a mean value of 18.8 γ per ml. and a range of 8 to 32.8 γ per ml. No explanation for patient variation could be discovered.

---

[15] Cycloserine was supplied as Seromycin® through the courtesy of Dr. R. S. Griffith and Dr. S. N. Gellis of Eli Lilly & Company.

[16] JOHNES, L. R.: Analytical Division Memo No. B-76, Commercial Solvents Corporation, July 9, 1954.

TABLE 1

REACTIONS TO CYCLOSERINE AND PYRAZINAMIDE

Toxic Symptoms	Number of Patients
Headache	12
Drowsiness	7
Nausea and anorexia	1
Somnolence	1
Joint pain	2

TABLE 2

CYCLOSERINE CONCENTRATION IN BLOOD (γ per ml.)

Number	Case	8 a.m.	2 p.m.	8 p.m.
1	A. K.	12.5	16.0	11.2
2	M. S.	13.7	16.4	12.5
3	E. G.	30.6	32.8	31.9
4*	V. B.	5.8	10.3	5.5
5	M. W.	16.1	23.9	15.6
6*	L. B.	20.7	25.2	19.4
7	C. R.	15.1	18.3	13.3
8	D. S.	6.6	8.0	5.1
9*	C. M.	17.8	20.8	15.8
10	R. G.	13.9	16.7	11.0
11*	H. R.	14.4	18.1	16.8
12*	E. A.	11.5	12.6	10.4
13	L. M.	8.8	15.7	9.0
14*	R. H.	18.9	19.8	18.0
15	B. T.	17.7	23.5	18.7
16*	E. E.	16.2	20.3	15.9
17*	D. R.	18.9	20.0	16.4
18	D. J.	16.0	18.9	14.7
19	C. S.	11.4	13.8	11.0
20*	B. M.	15.7	18.7	14.2
Mean		15.1	18.8	14.3

* Patients whose sputum became noninfectious.

TABLE 3

MEAN BLOOD CONCENTRATIONS OF CYCLOSERINE (γ per ml.)

Presence of Tubercle Bacilli in Sputum	8 a.m.	2 p.m.	8 p.m.
Tubercle bacilli disappeared (9 patients)	15.5	18.4	14.7
Tubercle bacilli persisted (11 patients)	14.7	18.5	14.0

No relationship between cycloserine concentration in the blood and the disappearance of tubercle bacilli from the sputum was apparent, but the total number of observations on this point is small. The mean values of cycloserine concentration in the blood related to reversal of infectiousness are shown in table 3.

Each patient had three concentrated sputum specimens examined by microscopy and culture each month while they were on treatment. When all three sputum specimens were negative for tubercle bacilli, they were recorded as negative. The individual results are recorded in table 4. Seven patients had sputum negative for tubercle bacilli on culture

TABLE 4

BACTERIOLOGIC STUDIES OF SPUTUM

Number	Case	1 Month		2 Months		3 Months		4 Months		5 Months		6 Months		
		M.	C.	M.	C.	M.	C.	M.	C.	M.	C.	M.	C.	
1	A. K.	−	+	+	+	+	+	+	+	+	+	+	+	
2	M. S.	+	+	−	+	−	+	+	+	+	+	+	+	
3	E. G.	+	+	−	−	+	−	+	+	+	+	+	−	
4*	V. B.	−	+	−	−	−	−	−	−	−	−	−	−	
5	M. W.	+	+	+	+	+	+	+	+	+	+	+	+	
6*	L. B.	−	+	−	+	−	−	−	−	−	−	−	−	Discharged
7	C. R.	+	+	+	+	+	+	+	+	+	+	+	+	
8	D. S.	+	+	+	+	+	+	+	+	+	+	+	+	
9*	C. M.	−	−	−	−	−	−	−	−	−	−	−	−	Discharged
10	R. G.	+	+	+	+	+	+	+	+	+	+	+	+	
11*	H. R.	+	−	−	+	−	−	−	−	−	−	−	−	Discharged
12*	E. A.	+	−	+	+	−	+	−	+	−	−	−	−	
13	L. M.	+	+	+	+	+	+	+	+	+	+	+	+	
14*	R. H.	−	−	−	−	−	−	−	−	−	−	−	−	Discharged
15	B. T.	+	+	−	−	+	+	−	−	−	−	+	−	AMA
16*	E. E.	+	−	−	−	−	−	−	−	−	−	−	−	Discharged
17*	D. R.	+	−	−	−	−	−	−	−	−	−	−	−	Discharged
18	D. J.	+	+	+	−	+	−	−	+	−	−	−	−	
19	C. S.	+	+	+	+	+	+	+	+	+	+	+	+	
20*	B. M.	−	−	−	−	−	−	−	−	−	−			AMA
Total with sputum negative for tubercle bacilli on microscopy and culture		6	7	11	9	10	10	11	9	11	11	9	11	

M = microscopy.
C = culture.
* = sputum became noninfectious.
AMA = left against medical advice.

TABLE 5

CYCLOSERINE SUSCEPTIBILITY OF PATIENT-STRAINS OF TUBERCLE BACILLI

	10 γ per Ml.	20 γ per Ml.	80 γ per Ml.
Prior to the treatment			
Resistant	20	18	0
Susceptible	0	2	20
After 6 months of treatment			
Resistant	20	20	2
Susceptible	0	0	18

after one month of therapy; 9, after two months; 10, after three months; 11 after five months; and 9, at six months. There were 9 patients whose sputum became negative and remained negative more than six months.

Six patients were discharged on the same therapy as had been received in the hospital. Two patients left the hospital against medical advice: one had been noninfectious for five months, the other would occasionally discharge tubercle bacilli throughout his stay. Follow-up data on the discharged patients revealed that 3 still had noninfectious sputum and were on the same treatment for more than one and one-half years. All had persistent cavities. One patient died from an accident and 2 were lost to follow-up study.

All of the strains of tubercle bacilli (table 5) were resistant to 10 γ of cycloserine per ml. prior to treatment. Only two patient-strains were susceptible to 20 γ of cycloserine per ml. After six months of treatment two patient-strains became resistant to 80 γ of cycloserine per ml.

It can be concluded that, even prior to treatment, tubercle bacilli are resistant to low concentrations of cycloserine *in vitro*.

Only 8 patients showed slight to moderate improvement by roentgenography at the end of six months of treatment. Nine patients showed no change and 3 patients became worse. Of the 9 patients whose sputum became negative for tubercle bacilli, 6 showed slight to moderate roentgenographic improvement and 3 showed no change. Four had persistent cavity with noninfectious sputum. Roentgenographic change was not a striking feature.

In summary: Twenty tuberculous patients who failed to respond to streptomycin, isoniazid, PAS, and viomycin, and in whom strains of tubercle bacilli resistant to these drugs emerged, were treated with cycloserine (250 mg. every twelve hours) and pyrazinamide (1 gm. three times daily) for six months or more. The toxicity of cycloserine when combined with pyrazinamide was very mild and the symptoms were limited to headache and drowsiness. These symptoms occurred within the first nine days and lasted for three to five days, after which they disappeared spontaneously.

Sixty per cent of the patients had sputum cultures negative for tubercle bacilli at the end of six months of treatment. The sputum of 9 of 20 patients remained negative for tubercle bacilli for more than three months continuously.

Eight of 20 patients showed slight to moderate roentgenographic improvement, 9 showed no change, and 2 became worse. It is believed that the use of a cycloserine-pyrazinamide regimen warrants further trial both for initial treatment and for re-treatment cases.

Ohio Tuberculosis Hospital and
The Ohio State University
Columbus, Ohio

Eizo Toguri
Robert J. Atwell

April 14, 1958

ZONE ELECTROPHORESIS IN STARCH GELS

A Report on Smithies Method in Normal Adults and in Patients with Tuberculosis

Zone electrophoresis of human serum in starch gels[1] has revealed new protein components derived from the $\alpha 2$ globulin which has proved to be a complex mixture of many different fractions.

Poulik and Smithies[2] have extended this work by using a combination of filter paper plus starch gel—two-dimensional electrophoresis—and with this they describe more than twenty serum protein components; at least ten can be recognized in the $\alpha 2$ globulin fraction. Certain of these $\alpha 2$ components combine with hemoglobin and appear to be identical with the haptoglobins previously studied by Jayle and Boussier.[3] By virtue of these haptoglobins, normal serum can be separated into three different types, initially described by Smithies as Type 1, Type 2*A*, and Type 2*B*, and later as Type 1-1, 2-1, 2-2. These serum types do not appear to be related to the blood groups (O, A, B, AB, Rh+, Rh−). Galatius-Jensen[4] has confirmed this serum typing in 1,033 unrelated persons. Allison and associates[5] have demonstrated a fourth type (O-O) in which no haptoglobins are present.

In view of the distinct changes in the $\alpha 2$ band following tuberculous infection and the new analysis of this same band by the starch gel technique, a study of this method on the serum of tuberculous patients appeared to be justified.

The aim was first to test Smithies' results in the serum from normal persons and, after this, use the one-dimensional technique to study the starch gel pattern in patients with varying degrees of tuberculous infection.

Certain problems were encountered with respect to the technique. A total of 666 gels were prepared, but only with the last 71 was a satisfactory and reproducible technique developed to permit examination of 142 serum samples, i.e., 2 serum specimens were examined simultaneously in each gel.

Despite adherence to the technique originally described by Smithies, the problems encountered fell into three categories:

Starch: Four commonly available starches (B.D.H. soluble starch Analar®, B.D.H. Amylum,® B.D.H. potato starch, and Hopkins and Williams potato starch) did not give satisfactory results. Eventually, a starch prepared by the Connaught Medical Research Laboratories, Starch-Hydrolysed® (Batch 101) proved to be satisfactory.

The buffer used for the gel: Increasing the initial pH of the borate buffer to 9.5 and adjusting the final pH of the gel to 8.6 produced a better separation of the globulin bands.[6] Later, using Poulik's[7] *tris*-citrate buffer (in place of the borate), satisfactory gel patterns were achieved.

The electrophoretic conditions: A locally constructed power pack made it possible to run three gels simultaneously in parallel. Unfortunately, with this system the potential fluctuated at different stages in the "run" and it was difficult to maintain a constant 6 volts

---

[1] Smithies, O.: Biochem. J., 1955, *61*, 629.
[2] Poulik, M. D., and Smithies, O.: Biochem. J., 1958, *68*, 636.
[3] Jayle, J. F., and Boussier, G.: Exp. Ann. Biochim. Med., 1955, *17*, 157.
[4] Galatius-Jensen, F.: Acta genet., 1957, *7*, 549.
[5] Allison, A. C., Blumberg, B. S., AP Rees, W.: Nature, London, 1958, *181*, 824.
[6] Harris, H.: Personal communication, 1957.
[7] Poulik, M. D., Nature, London, 1957, *180*, 1477.

per cm. across each gel. These difficulties were later overcome by the use of heavy silver/silver chloride (Tiselius pattern) electrodes and the introduction of a double bridge solution.

Normal adults: One hundred twenty-four experiments were made on 65 normal subjects and the serum type of distribution was as follows: Type 1-1—5 (8 per cent); Type 2-1—40 (61 per cent); Type 2-2—20 (31 per cent).

Patients with tuberculosis: Serum samples were obtained from 25 inpatients either before treatment was started or within the first month of chemotherapy. Tubercle bacilli were isolated from all except 2. There were 22 patients with pulmonary tuberculosis (one minimal, 8 moderately advanced, and 13 advanced), one patient with a tuberculous cervical adenitis, and 2 with skeletal tuberculosis. The serum pattern of the tuberculous patients did not differ from that of the normal subjects apart from minor changes in the length of the albumin band. No specific pattern was demonstrated in the different $\alpha 2$ globulin fractions despite the five component bands. These results were further checked by running the tuberculous serum against a normal one in the same gel. The serum type of distribution among these patients was: Type 1-1—0; Type 2-1—18 (72 per cent); Type 2-2—7 (28 per cent).

The failure to demonstrate any qualitative change in the $\alpha 2$ fractions of tuberculous serum would indicate that the decrease in the total $\alpha 2$ globulin shown by paper electrophoresis affects all of the fractions demonstrated in one-dimensional starch gel electrophoresis. In the limited number of patients studied, it is probably that there is no one serum type more prone to tuberculous infection. Poulik and Smithies,[2] using the later two-dimensional technique, studied eight abnormal serum specimens but none from cases of tuberculosis. The present writers have not had an opportunity to examine this more elaborate modification and it is possible that this method will evolve yet further.

In summary: A new technique for analyzing the $\alpha 2$ globulin fraction of the serum (one-dimensional starch gel electrophresis) has revealed no specific qualitative change in serum specimens from 25 patients with active tuberculosis.

Department of Tuberculosis
University of St. Andrews
Dundee, Scotland

R. N. Johnston[a]
I. Steven

June 23, 1958

[a] This work was carried out during the tenure of a grant held from the Scottish Hospital Endowments Research Trust and from the Board of Management for the Dundee General Hospitals.

The writers gratefully acknowledge the help of Dr. J. M. Stowers, who introduced them to this technique; of Professor R. B. Hunter and Dr. R. P. Cook for facilities in their laboratories; of Dr. C. Cameron of the Blood Transfusion Service; and of Mr. F. Steven, Mr. D. Allan, and Mr. H. McDonald for technical help.

THE CULTIVATION OF THE BACILLE-CALMETTE-GUERIN STRAIN OF M. TUBERCULOSIS (BCG)

In 1956, Dubos and his associates reported a series of experiments characterizing under experimental conditions in mice the degree of immunity achieved with various strains of living BCG.[1-4] Subsequently, Weiss and Dubos called attention to the fact that cell-free methanol extracts of such organisms are likewise capable of protecting against tuberculous infection in mice, but fail to confer hypersensitivity to tuberculoprotein.[5, 6] These observations were later extended in guinea pigs by Weiss, who clearly demonstrated that the degree of protective immunity produced by such extracts is approximately one-half as great as that produced by living BCG, but may be enhanced by the simultaneous administration of Freund's adjuvant.[7]

Developments during recent years in the chemotherapy of tuberculosis have led to general recognition of the fact that mechanisms for increasing host resistance must be developed before true eradication of the infection will be achieved. The simultaneous gradual decrease in incidence of tuberculin-positive individuals among the general population in certain areas has led to realization of the potential practical value of the tuberculin reaction in the detection of infected persons. Thus, further study of extracts or other agents capable of conferring protective immunity without simultaneously producing skin hypersensitivity to tuberculoprotein are warranted. Such studies are in progress in a number of laboratories.

The ability to cultivate *Mycobacterium tuberculosis, en masse*, is of basic importance in the development of cell-free immunizing agents therefrom. The present study was undertaken, therefore, to evaluate conditions which will allow easy and inexpensive cultivation of large numbers of mycobacterial cells.

The Phipps (or Aronson) strain of BCG[8] was used in all of the experiments which will be described herein.

Stock cultures were maintained in all instances in the conventional Tween®-albumin liquid medium. Seed cultures were obtained by routinely inoculating 0.1 ml. of a stock culture into 25 ml. of the same medium (in a 250-ml. Erlenmeyer flask), and incubating for a period of seven days (37° C.) under stationary conditions. Cultures to be used as inoculum for larger volumes of medium were then prepared by inoculating 6 ml. of the above-described seed culture into 100 ml. (in a 500-ml. Erlenmeyer flask) of the antigenic extract medium described by Weiss and Dubos in 1955.[5] Again cultures were incubated for seven days (37° C.) under stationary conditions. One hundred milliliters of inoculum, prepared as described, were then used to seed each 1,000 ml. (in a 2,800-ml. Fernbach flask) of the special media devised for production of large quantities of organisms. At this stage, all cultures were incubated at 37° C. for varying periods of time on a rotary shaker[9] set at approximately

---

[1] Dubos, R. J., and Pierce, C. H.: Am. Rev. Tuberc., 1956, *74*, 655.

[2] Pierce, C. H., and Dubos, R. J.: Am. Rev. Tuberc., 1956, *74*, 667.

[3] Pierce, C. H., *et al.*: Am. Rev. Tubere., 1956, *74*, 683.

[4] Dubos, R. J., and Pierce, C. H.: Am. Rev. Tuberc., 1956, *74*, 669.

[5] Weiss, D. W., and Dubos, R. J.: J. Exper. Med., 1955, *101*, 313.

[6] Weiss, D. W., and Dubos, R. J.: J. Exper. Med., 1956, *103*, 73.

[7] Weiss, D. W.: Am. Rev. Tuberc., 1958, *77*, 719.

[8] Received from Dr. René Dubos of the Rockefeller Institute for Medical Research, New York 21, New York (1–4).

[9] The rotary shaker used at this stage of the study was manufactured at Chas. Pfizer & Co., Inc. More recently, a New Brunswick Rotary Shaker (Model No. G52) has been used with satisfactory, although somewhat lower, cell yields, i.e., 10 gm. of lyophilized cells per liter of medium.

200 r.p.m. A total of 31 modifications of Sauton, Long, and the conventional Tween-albumin liquid media were tested for their ability to support growth of the organism.

Medium: Optimal results were obtained in a synthetic medium having the following composition:

Sodium glutamate (CP)	50 gm.
Citric acid (hydrous, CP)	20 gm.
Glycerol (CP)	600 gm.
$K_2HPO_4 \cdot 3H_2O$(CP)	5 gm.
Ferric ammonium citrate (i.e., ferric and ammonium citrates, brown, USP, 17.5% iron, 8.7% ammonia)	0.5 gm.
$MgSO_4 \cdot 7H_2O$ (CP)	5 gm.
Distilled H_2O, to:	10 liters
NH_4OH, to:	pH 7.2

Less satisfactory results were obtained when crude reagents were substituted for CP or USP chemicals. Moreover, less satisfactory results were obtained when asparagine, ammonium succinate, N-Z Case (Sheffield), or $(NH_4)_2HPO_4$ were substituted for sodium glutamate in the medium, and/or when glucose was used to replace glycerol. In addition, lower yields resulted in the absence of ferric ammonium citrate. The conventional Sauton, Long, and Tween-albumin media were inferior under the conditions employed in this series of experiments.

Incubation period and yields obtained: Maximal yields of bacterial cells were obtained in the sodium glutamate-glycerol medium described above when cultures were incubated at 37° C. either for twelve days with manual shaking once daily followed by eleven days on the rotary shaker (*see* above), or for fourteen days on the shaker with no previous period of stationary growth. Using this incubation period, the yield of acetone-washed and air-dried, or water-washed and lyophilized, phenol-killed cells, in 12 recent consecutive experiments, ranged from 9.1 to 28.8 (average 19.45) gm. per liter of medium (table 1). The quantity of cells increased most rapidly during the early portion of the incubation period, i.e., during the first four days of shaking, but continued to increase to some extent thereafter (table 2).

The number of viable cell units at the end of the above-mentioned incubation period ranged generally from 128 to 132 million. Thus, the number was approximately the same as that observed in this laboratory with the use of the conventional Tween-albumin medium under stationary conditions after an incubation period of eight to ten days. One may assume, therefore, that many nonviable cells conceivably may be present in the sodium glutamate-glycerol medium at the end of the incubation period.

Morphology: In the experiments conducted to date, BCG cells grown under submerged conditions, according to the technique described, appeared as elongated, slender, pale staining acid-fast rods with large, deeply-staining metachromatic granules. A portion of the cells appeared singly; the majority were closely bound in parallel formation. When subcultured onto oleic-acid–albumin agar, the colonies were typical but extremely small. Impression smears of these colonies, when stained by the Ziehl-Neelsen technique, showed organisms morphologically indistinguishable from those grown under stationary conditions and subsequently subcultured onto oleic-acid–albumin agar, i.e., they were short, somewhat blunt acid-fast rods in parallel formation with few, if any, metachromatic granules.

Effects in vivo: BCG cells (Phipps strain), when cultivated under submerged conditions as described above, and subsequently inoculated subcutaneously into guinea pigs, within two to three weeks after inoculation produced large local lesions which later regressed.

TABLE 1

YIELDS OF DRIED PHENOLIZED CELLS (PHIPPS STRAIN, BCG)

Lot Number	Grams per Liter
050157	23.4
071057	9.1
091857	19.7
100957	18.4
102257	15.6
102357	18.3
103057	26.1
110657	23.8
111357	28.8
112757	15.2
120457	19.2
121157	15.8
Average yield	19.45

TABLE 2

LENGTH OF INCUBATION PERIOD VERSUS WEIGHT OF PHENOLIZED DRIED CELLS (Average Yield Obtained with 3 Lots)

Incubation Time (days)		Yield	Number-fold Increase in Weight
Stationary	Shaker		
		gm.	
12	0	0.12	
12	4	2.02	16.8
12	7	10.39	5.1
12	11	16.82	1.6

As illustrated in figure 1, mice infected intravenously with these cells died rapidly. In a single experiment, for example, 9 of 20 animals receiving 13.2 million viable cell units intravenously died within forty-eight hours. Two of the remaining 11 died four to six days after challenge, while 8 of the 11 died between eleven and twenty-five days after challenge. Tubercle bacilli were recovered from the lungs of all of these last-mentioned 11 animals. Ten of the 11 were examined histologically.[10] Nine showed an extensive organizing tuberculous pneumonia; one showed only moderate numbers of cellular foci.

Essentially all mice, simultaneously challenged intravenously with smaller numbers of shaker-grown cells (1.32 and 0.132 million viable cell units per mouse) or with larger numbers of cells (11 to 13 million viable cell units) grown in the conventional Tween-albumin liquid medium under stationary conditions, survived for a period of 119 days after challenge. When sacrificed at this time they showed only cellular foci in the lungs and at times in the kidneys (*see* figure 1).

[10] The writers are indebted to Dr. Oscar Auerbach of the East Orange (New Jersey) Veterans Administration for all of the histologic readings.

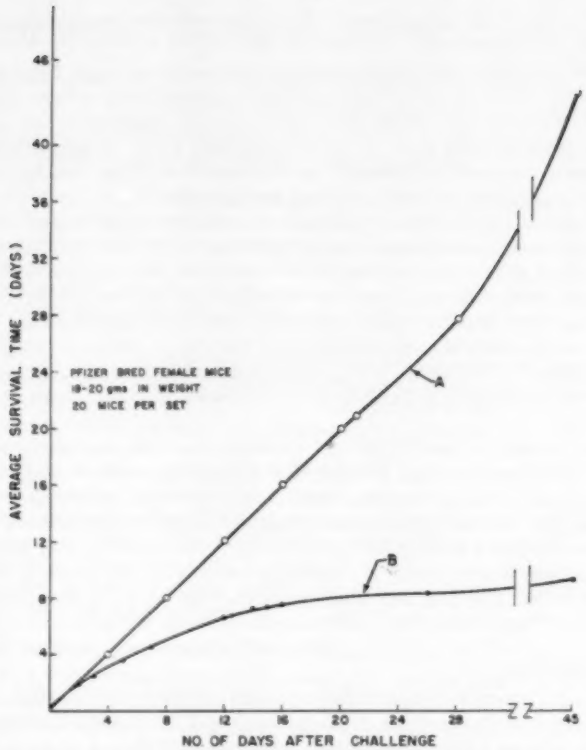


FIG. 1. Average survival time of *all* mice, calculated at indicated time (days) following intravenous challenge with the Phipps strain of BCG. *A:* challenged with eight-day culture, stationary growth, Tween-albumin medium; challenge dose equivalent to 11.2 million viable cell units. *B:* challenged with eleven-day culture, rotary shaker, sodium glutamate; challenge dose equivalent to 13.2 million viable cell units.

The data presented herein establish the fact that high yields of BCG cells may be obtained with ease by cultivation under submerged conditions in a sodium glutamate-glycerol medium. That tubercle bacilli can grow and multiply under submerged conditions is not a new observation.[11] In so far as the writers are aware, however, such high yields of mycobacterial cells have not previously been obtained in such a short period of incubation.

Not all of the cells present in cultures grown according to the technique described herein are viable. The exact ratio of viable to nonviable cells, moreover, may be of importance, for it is not inconceivable that a lesser weight of viable organisms might be more suitable for extraction of the substance(s) which confer protective immunity than larger quantities of nonviable cells. Attempts to determine the exact proportion of viable to nonviable cells,

[11] DREA, W. F., AND ANDREJEW, A.: The Metabolism of the Tubercle Bacillus, Chas. C Thomas, Springfield, Illinois, 1953, pp. 67–118.

however, have thus far been unsuccessful, due to their cohesive nature when grown in this manner.

Of interest is the fact that sodium glutamate appears to be largely responsible for the yields obtained. Ginsburg and his associates[12] have reported previously on this point and have presented evidence to indicate that L-glutamic acid, DL-glutamic acid, and L-glutamaine were more effective than 31 other compounds tested in promoting growth of Mycobacteria in the absence of other nitrogen sources. In addition, they have shown that the glutamic acid content of certain virulent strains is high.[12]

It has been clearly established by studies reported by Dubos and Schaedler in 1957,[14, 15] and by additional experiments conducted in this laboratory,[16] that BCG cells grown in the manner described in this report are capable of conferring protective immunity, at least in mice. Whether or not maximal amounts of the significant fraction can be obtained from such cells remains to be determined. It is possible that more virulent strains of *Mycobacterium tuberculosis* might be more satisfactory in this respect, but no evidence is available as yet to indicate the extent to which virulent strains will grow and multiply under the conditions selected in this study as optimal for cultivation of BCG. Studies on this subject are in progress.

The observations presented herein, with respect to the death rate among animals challenged with BCG grown under submerged conditions in a sodium glutamate-glycerol medium, in no way suggest that this strain of Mycobacteria has increased in virulence during cultivation. Indeed, Dubos and Schaedler,[14, 15] as well as Rzucidlo and Garbinski,[17] have clearly established in mice that phenol-killed BCG, simultaneously injected by the intravenous route with a strain of *Staphylococcus aureus*, can interfere with clearing mechanisms within the host and thereby increase the animal's susceptibility to the Staphylococci. The probably high proportion of nonviable to viable cells in the sodium glutamate-glycerol cultures of BCG, as illustrated in table 2, may account, therefore, for the results observed.

In summary: Data have been presented to indicate that BCG may be cultivated with ease, in large quantity, in short periods of time. Background information has been presented, moreover, concerning certain of the biologic properties of BCG cells grown in this manner.

RESEARCH DIVISION
CHAS. PFIZER & CO., INC.
630 FLUSHING AVENUE
BROOKLYN 6, NEW YORK

TULITA F. LENERT
IRENE STASKO
GLADYS L. HOBBY

June 25, 1958

[12] GINSBURG, B., AND DUNN, M. S.: Am. Rev. Tuberc., 1957, *75*, 688.
[13] GINSBURG, B., *et al.*: Arch. Biochem., 1956, *60*, 164.
[14] DUBOS, R. J., AND SCHAEDLER, R. W.: J. Exper. Med., 1957, *106*, 703.
[15] SCHAEDLER, R. W., AND DUBOS, R. J.: J. Exper. Med., 1957, *106*, 719.
[16] HOBBY, G. L., *et al.*: Am. Rev. Tuberc., 1958, *78*, 939.
[17] RZUCIDLO, L. T., AND GARBINSKI, T.: Am. Rev. Tuberc., 1958, 77, 1026.

THE BIOLOGIC ACTIVITY OF CRUDE EXTRACTS OF BCG

In a previous communication, a technique for the cultivation of the Bacille-Calmette-Guerin strain (BCG) of *M. tuberculosis, en masse*, was described.[1] In the present report, data will be presented to illustrate the extent to which such cells, or crude extracts thereof, are capable of protecting against tuberculous infection in mice.

In the present study, the Bacille-Calmette-Guerin strain of *M. tuberculosis* was used throughout for preparation of immunizing agents. In all instances, the BCG cells were grown in the manner previously described.[1] Cultures were phenolized at the end of the incubation period, and the cells were removed seventy-two hours later by centrifugation.[2] In initial experiments, the cells were then acetone-washed and dried in air at room temperature; in later experiments they were washed with distilled water only and dried by lyophilization.

Crude extracts of the phenolized dried BCG cells were prepared by suspension of the cells in distilled water and by their subsequent rupture by sonic oscillation.[3] At the end of the treatment period (two hours at maximal power), a few intact cells remained among masses of granular debris. All extracts were stored in the frozen state and were thawed once only, at the time of use.

Both the above-mentioned whole cells and sonic extracts derived from such cells were utilized as immunizing agents in the experiments which will be described herein. In all instances, mice were immunized by the subcutaneous route, either a single protective dose being used or two such doses, administered seven days apart. Animals were challenged routinely by the intravenous route, with the Vallée (bovine) strain of *M. tuberculosis*, either three weeks after injection of a single immunizing dose or two weeks after injection of the second of two such immunizing doses.

Protective action of crude BCG cells: A total of 35 batches of crude (phenolized, dried) BCG cells were tested for their ability to protect mice against challenge with the Vallée strain of *M. tuberculosis*. Marked variation in the effect produced by the various batches was noted. In some instances, the protective effect was readily apparent; in other instances, the effect was slight. At times, as previously observed by Dubos and his associates,[4] more marked protection was observed with lower, than with higher, doses. The protective action of the BCG cells manifested itself, moreover, by an initial delay in death time more often than by a decrease in over-all death time (figure 1).

Protective action of sonic extracts of BCG: Greater protection at times was obtained by the subcutaneous administration of BCG cells ruptured by sonic vibration than by injection of equal quantities of whole cells (figure 2). Moreover, the protective effect was enhanced by administration of the BCG cells, or extract thereof, in Freund's adjuvant (figure 3). The effect observed with this adjuvant, however, was no greater than that achieved by administration of two doses of the immunizing agent without adjuvant (figure 3).

Nonspecificity of BCG as compared with certain bacterial endotoxins: Using acute infections produced by intraperitoneal challenge with *D. pneumoniae* (strain I/230) and *Staphylococcus aureus* (strain ℘NYH235), the ability of crude phenolized BCG cells to protect nonspecifically was demonstrated. This has previously been shown by Dubos

[1] LENERT, T. F., *et al.*: Am. Rev. Tuberc., 1958, *78*, 934.

[2] Sharples Centrifuge.

[3] A Raytheon Sonic Oscillator, Model No. DF101, was used for this purpose.

[4] DUBOS, R. J., AND SCHAEDLER, R. W.: J. Exper. Med., 1957, *106*, 703.

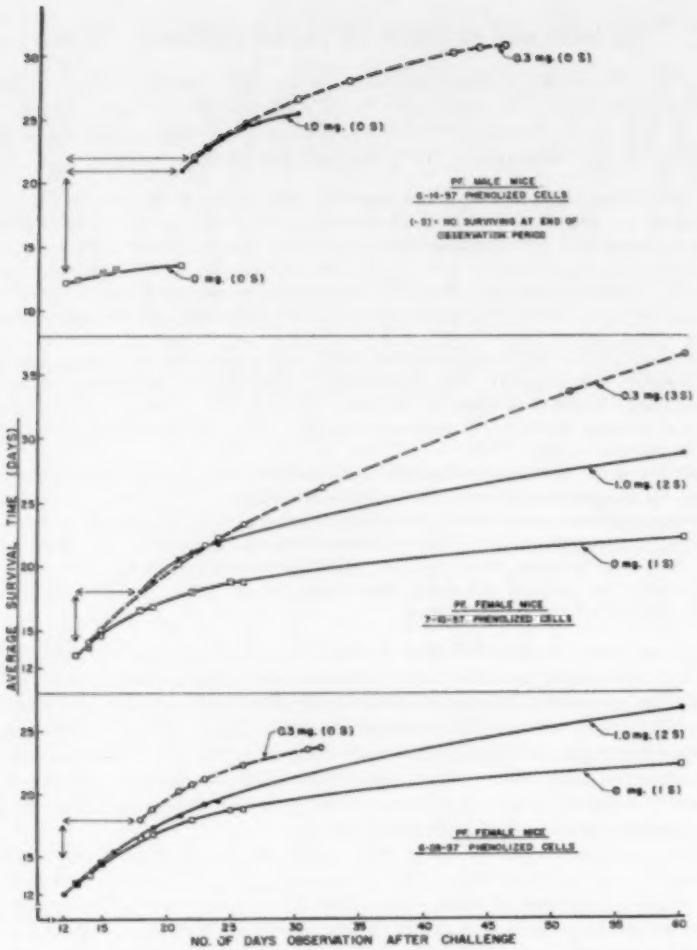

0.3 mg. (0 S)
1.0 mg. (0 S)
0 mg. (0 S)
PF. MALE MICE
6-10-57 PHENOLIZED CELLS
(-S) = NO. SURVIVING AT END OF OBSERVATION PERIOD
0.3 mg. (3 S)
1.0 mg. (2 S)
0 mg. (1 S)
PF. FEMALE MICE
7-10-57 PHENOLIZED CELLS
0.3 mg. (0 S)
1.0 mg. (2 S)
0 mg. (1 S)
PF. FEMALE MICE
6-28-57 PHENOLIZED CELLS
AVERAGE SURVIVAL TIME (DAYS)
NO. OF DAYS OBSERVATION AFTER CHALLENGE

FIG. 1. Average survival time of mice immunized with 3 representative batches of BCG cells three weeks prior to intravenous challenge with the Vallée strain of *M. tuberculosis*. The average survival time was determined by averaging the duration of survival of *all* animals in each series up to the indicated time (days after challenge) or observation point.

and associates,[4] and by others. In the present experiments, however, the average survival time of animals infected after immunization with BCG cells, or fractions thereof, was never so long as that of infected animals previously injected with Piromen® or a lipopolysaccharide fraction derived from *A. aerogenes*[5] (table 1).

[5] Piromen is a mixture of nucleic acids and a polysaccharide complex that contains lipid and nitrogen attributable to chemically bound glucosamine.[6] It is derived from *Pseudo-*

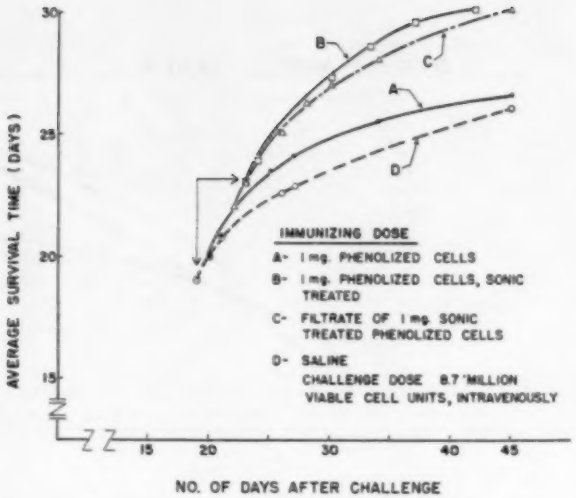


FIG. 2. Average survival time of mice immunized with phenolized BCG and with sonic extracts and filtrates thereof, after challenge with the Vallée strain of *M. tuberculosis*. The average survival time was determined by averaging the duration of survival of *all* animals in each series up to the indicated time (days after challenge) or observation point.

It is a well-known fact that variations in cultural conditions may alter the constituents of microbial cells. The possibility, therefore, that the BCG strain of *M. tuberculosis*, when cultivated under submerged conditions, might not contain the substance(s) necessary to confer immunity upon experimental animals warranted careful consideration. The data presented herein offer abundant evidence, however, to indicate that cells grown in this manner are capable of protecting against tuberculous infection in mice. The results thus confirm the earlier observations of Weiss and Dubos[7,8] and of Weiss,[9] made with cells grown in a more conventional manner.

It was previously observed by Dubos and his associates[4] that phenolized BCG cells contain not only a protective agent or agents, but also one or more substances capable of increasing host susceptibility. This is again emphasized by the marked variation in protective power of the various batches tested in the present study and by the greater protective effect achieved at times with lower, in contrast to higher, quantities of cells or of cell extracts.

---

monas aeruginosa and is marketed under the name of Piromen by Travenol Laboratories, Inc., Morton Grove, Illinois. The lipopolysaccharide fraction of *A. aerogenes* used in the present studies was furnished to us by Dr. R. J. Reedy of the Food and Drug Administration, Washington, D. C.

[6] GINGER, L. G.: See Chapter XV by Stuart, E. G.: Tissue reactions and possible mechanisms of Piromen and desoxycorticosterone acetate in central nervous regeneration in *Regeneration in the Central Nervous System*, Charles C Thomas, Springfield, Illinois, 1955.

[7] WEISS, D., AND DUBOS, R. J.: J. Exper. Med., 1955, *101*, 313.

[8] WEISS, D., AND DUBOS, R. J.: J. Exper. Med., 1956, *103*, 73.

[9] WEISS, D.: Am. Rev. Tuberc., 1958, *77*, 719.

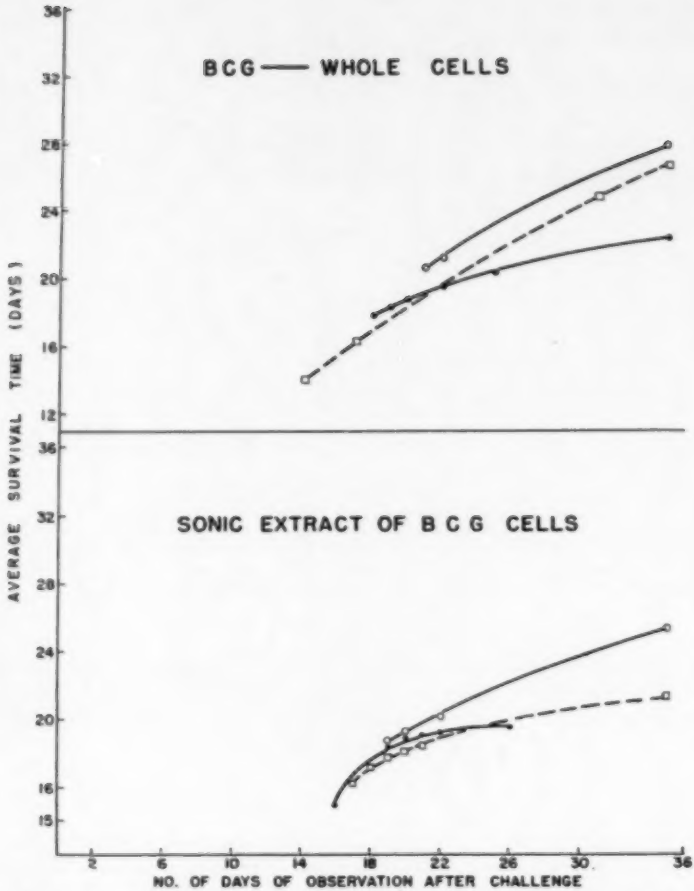


FIG. 3. Average survival time of mice immunized with phenolized BCG cells and extracts thereof three weeks prior to challenge with the Vallée strain of *M. tuberculosis*. The average survival time was determined by averaging the duration of survival of *all* animals in each series up to the indicated time (days after challenge) or observation point. *Upper section:* whole cells used as immunizing agent; *lower section:* sonic extracts of cells used for immunization. ●——● single immunizing dose, equivalent to 1 mg. of cells; ○——○ two immunizing doses, administered seven days apart, and each equivalent to 1 mg. of cells; □---□ single immunizing dose, equivalent to 1 mg. of cells, administered in 0.05 ml. of Freund's adjuvant.

The fact that rupturing the cell may at times release larger amounts of the protective substance may be of practical importance. Preliminary studies have suggested, moreover, that this substance may pass through certain of the commonly used microbial filters.

The present observations further confirm those of Dubos and his associates[7, 8] and of Weiss,[9] in which they clearly showed that the protective effect may be enhanced by the

TABLE 1

THE NONSPECIFIC PROTECTIVE ACTION OF CRUDE PHENOLIZED BCG CELLS

Immunizing Dose Administered Subcutaneously 24 Hours Before Intraperitoneal Challenge with *D. pneumoniae* (I/230)

Immunization		Average Survival Time in Hours Evaluated at 10 Days
Agent	Dosage	
Saline	0.1 ml.	46.5
BCG cells: #010657	100 γ	61.3
BCG cells: #061457	100 γ	65.2
Methanol extract of BCG (#10)*	100 γ	90.2
#114 A. aerogenes pyrogen*	10 γ	118.9
Piromen	10 γ	145

* Methanol extract #10 was prepared by Dr. R. Schaedler of the Rockefeller Institute for Medical Research from cells comparable to those described herein. Preparation #114A was received from Dr. R. J. Reedy of the Food and Drug Administration, Washington, D. C.

simultaneous administration of Freund's adjuvant. No data are available as yet, however, with respect to the effectiveness of other more readily tolerated adjuvants. Nor are data available to indicate whether or not a greater effect would be achieved by the use of phenolized virulent cells or cell-free extracts thereof. The fact that multiple (two) doses of the immunizing agents employed herein resulted in a protective effect equal to that achieved by administration of a single dose in Freund's adjuvant suggests the possibility, however, that careful evaluation of immunizing regimens may give optimal results without resort to virulent cells as source material.

All preparations tested during the present study were capable of protecting to some degree at least against challenge with certain nonacid-fast microorganisms. In the dosages used, none increased the average survival time of infected animals to as great an extent as did certain gram-negative endotoxins; yet none were as highly purified preparations as were the latter substances. All contained tuberculoprotein, as would be anticipated, and as evidenced also by sensitization studies conducted in guinea pigs.

In summary: Data have been presented which indicate that BCG cells, grown under submerged conditions and subsequently phenolized, are capable of protecting against tuberculous infection in mice. Moreover, evidence was presented which suggests that cells ruptured by sonic oscillation may offer greater protection than crude whole cells. The protective effect in most instances was significantly enhanced by the simultaneous administration of Freund's adjuvant or by the administration of multiple doses of the immunizing agent.

RESEARCH DIVISION
CHAS. PFIZER & CO., INC.
630 FLUSHING AVENUE
BROOKLYN 6, NEW YORK

GLADYS L. HOBBY
TULITA F. LENERT
IRENE STASKO

June 25, 1958

COMPARISON OF ISONIAZID CONCENTRATIONS IN THE BLOOD OF PEOPLE OF JAPANESE AND EUROPEAN DESCENT

Therapeutic and Genetic Implications

Studies of the metabolism of isoniazid by Hughes[1, 2] indicate a significant difference in the metabolic fate of this drug in various animal species and among different human subjects. In man, essentially all of the isoniazid administered orally is excreted in the urine as the unchanged compound or as its metabolic products. The proportion of free isoniazid excreted in the urine has been shown to vary directly with the plasma concentration of the free drug.[3] Individuals differ widely in the concentration of biologically active (free) isoniazid obtained in the serum following a standard oral dose[4-6]; however, a single individual demonstrates little or no difference in the serum concentration upon repeated testing over periods of weeks to months.[5] Based on the rate of isoniazid degradation, Mitchell[7] divides subjects into "rapid inactivators" (0.2 γ or less per ml. of serum six hours following a standard test dose), "slow inactivators" (0.8 γ per ml. or higher at six hours), and "intermediate inactivators" (0.4 γ per ml. at six hours). He also noted that the pattern of isoniazid metabolism is not related to age or sex, nor is it influenced by the presence of pulmonary tuberculosis, silicosis, pulmonary emphysema, diabetes mellitus, renal insufficiency, or hepatic cirrhosis.[7] Evidence has been presented[6-8] which suggests that the results of treatment with isoniazid in patients with pulmonary tuberculosis may depend in part upon the concentrations of biologically active drug in the blood. To date, however, there is no published study of the underlying cause for this wide variation in the degree of biochemical inactivation of isoniazid in different individuals.

The present study was undertaken to compare the concentration of biologically active isoniazid in the blood of subjects from two different ethnic backgrounds. Twenty-five subjects of Japanese descent were studied—all were of the first generation born in the United States (Nisei). As definitely as could be determined, each subject was entirely of Japanese ancestry. With the exception of two siblings, the subjects were not blood relatives. The group was made up of healthy, active men and women ranging in ages from nineteen to forty-two years, employed as students, physicians, nurses, technicians, and other hospital workers. Their diet and home environment was typically American and did not differ from those of the control group. The other group was of European descent and was made up of 25 individuals of essentially similar ages, sex distribution, and occupation. No blood relatives were included. These subjects were all American-born but were of diverse European ancestry. Mixtures of Anglo-Saxon, Scandinavian, or northern European origin were represented. Individuals of southern, eastern European, or Asian ancestry

---

[1] HUGHES, H. B.: J. Pharmacol. & Exper. Therap., 1953, *109*, 444.

[2] HUGHES, H. B., *et al.*: Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 217.

[3] BIEHL, J. P.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 279.

[4] MORSE, W. C., *et al.*: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 283.

[5] BELL, J. C., AND RIEMENSNIDER, D. K.: Am. Rev. Tuberc., 1957, *75*, 995.

[6] KASS, I. *et al.*: Ann. Int. Med., 1957, *47*, 744.

[7] MITCHELL, R. S., AND BELL, J. C.: New England J. Med., 1957, *257*, 1066.

[8] RUSSELL, W. F., JR., AND MIDDLEBROOK, G.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 274.

were not included in the group. Each of the 50 subjects was ambulatory and none was shown to be ill or taking drugs.

The assays for biologically active isoniazid were determined as follows: At six a.m. the subject took isoniazid tablets orally; the dose, calculated to the nearest 25 mg., was 4 mg. per kg. of body weight. Blood samples were collected aseptically at two, four, and six hours

TABLE 1

CONCENTRATIONS OF BIOLOGICALLY ACTIVE ISONIAZID IN THE SERUM

Subjects of Japanese Descent				Subjects of European Descent			
	Serum Concentration (γ per ml.)				Serum Concentration (γ per ml.)		
	2 hours	4 hours	6 hours		2 hours	4 hours	6 hours
"Slow Inactivators"				"Slow Inactivators"			
Males	3.2	1.6	1.6	Males	3.2	1.6	0.8
	3.2	1.6	1.6		3.2	1.6	1.6
	3.2	3.2	1.6		3.2	1.6	0.8
					3.2	1.6	0.8
Mean	3.2	2.1	1.6		3.2	1.6	1.6
					3.2	3.2	1.6
"Rapid Inactivators"							
Males	1.6	0.4	0.2		1.6	0.8	0.8
	0.8	0.2	0.0		1.6	1.6	0.8
	0.8	0.2	0.0		3.2	1.6	1.6
	0.8	0.0	0.0		3.2	1.6	1.6
	0.4	0.0	0.0		1.6	0.8	0.4
	0.8	0.0	0.0				
	0.8	0.4	0.0	Females	3.2	1.6	0.8
	0.2	0.0	0.0		1.6	0.4	0.4
	1.6	0.4	0.2				
	0.8	0.0	0.0	Mean	2.7	1.5	1.1
	0.4	0.0	0.0				
	0.8	0.4	0.0	"Rapid Inactivators"			
	0.8	0.4	0.0	Males	0.8	0.4	0.0
	1.6	0.4	0.2		0.8	0.4	0.2
	0.8	0.4	0.2		1.6	0.8	0.2
	0.8	0.0	0.0		1.6	0.4	0.2
					1.6	0.8	0.2
Females	0.8	0.0	0.0		1.6	0.4	0.2
	0.8	0.2	0.0		0.8	0.4	0.0
	1.6	0.4	0.2		1.6	0.4	0.2
	0.4	0.0	0.0				
	0.8	0.0	0.0	Females	0.8	0.2	0.0
					0.8	0.2	0.0
					1.6	0.8	0.2
					1.6	0.2	0.0
Mean	0.87	0.17	0.05	Mean	1.4	0.47	0.13
Mean for the group	1.18	0.42	0.23	Mean for the group	1.95	0.95	0.6

after the test dose. Small amounts of water were allowed; the only food allowed was black coffee and dry toast with jelly at 8:15 a.m., after the two-hour blood specimen had been obtained. Serum from each of the blood specimens was used to prepare twofold dilutions in 5 ml. of 7-H-9 liquid medium. A control was prepared for each series of tests; this contained twofold serial dilutions of isoniazid ranging between 0.1 γ per ml. and 0.005 γ per ml. The minimal inhibiting concentration for the test organism was invariably 0.04 γ per ml. All tubes were inoculated with 0.05 ml. of a seven- to ten-day-old culture of *M. tuberculosis*, strain H37Rv (streptomycin resistant). The inoculum was standardized for each series of tests by diluting the culture to 60 per cent transmittance at a wave length of 460 μ Rouy-Leitz photoelectric colorimeter. All tests were incubated at 37°C. for fourteen days. The growth of *M. tuberculosis* in each tube was compared macroscopically with the control tube. Tubes containing no turbidity, or very slight turbidity (significantly less than that in the control tube), were recorded as negative. Subcultures were performed if contamination was suspected. The serum isoniazid concentration was determined by comparing the highest dilution of serum inhibiting growth with the minimal inhibiting concentration of isoniazid in the control tubes.

The serum concentrations at two, four, and six hours after ingestion of isoniazid are shown in table 1. The mean values are as follows:

	Japanese-Descent Subjects	European-Descent Subjects	Difference	P Value
2 hours	1.18	1.95	0.77	<0.001
4 hours	0.42	0.95	0.53	0.01
6 hours	0.23	0.6	0.37	<0.001

The observed difference between the mean values of isoniazid serum concentrations for the two groups is statistically significant. In figure 1, the subjects are divided into "slow," "rapid," and "intermediate" inactivators. Twenty-two of the 25 Japanese-descent subjects were rapid inactivators, whereas only 12 of the 25 of European descent fell into this group. This difference is also statistically significant (P value = 0.001). The mean serum concentrations of rapid and slow inactivators (including 2 of the intermediate inactivators) for both groups are illustrated in figure 2. For both groups the difference between the mean serum concentrations of the rapid inactivators and that of the slow inactivators is statistically significant at each of the three periods of measurement.

Morse,[9] working in Japan, discovered the average isoniazid concentration (biologically active) in the blood of native Japanese females to be much lower than that in non-Japanese. The data cited earlier in table 1 confirm Morse's observations and indicate a significant difference in the values for free isoniazid in Americans of Japanese descent as compared with those of European descent. This difference would appear to be related solely to the ethnic difference. The similarity of dietary and environmental factors in the two groups reported here tends to exclude these factors as possible causes for the observed difference.

The biochemical cause for the low drug concentrations in the majority of the Japanese-descent group is not known; it could be due to incomplete intestinal absorption, rapid urinary excretion, or rapid metabolic degradation of the drug. Analysis of the chemical fractions in the urine of non-Japanese rapid inactivators indicates that in this group the drug is largely converted into the biologically inactive acetyl derivative.[1] The urinary excretion of isoniazid and its metabolites in persons of Japanese descent is presently under

[9] MORSE, W. C.: Personal communication.

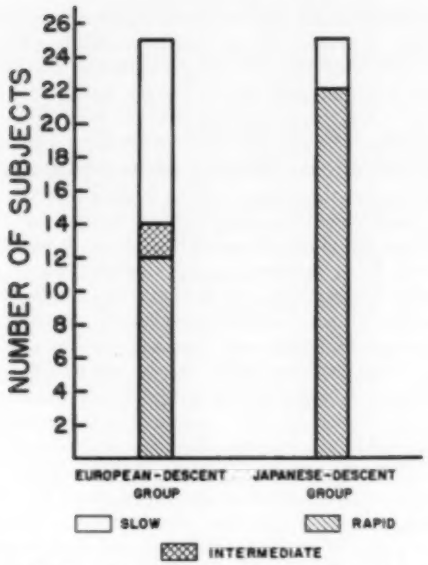


FIG. 1. The number of "slow," "rapid," and "intermediate" inactivators among 25 persons from each ethnic group, based on the six-hour serum concentration of biologically active isoniazid.

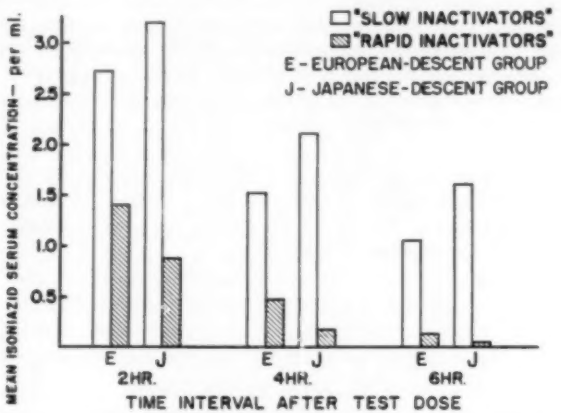


FIG. 2. The mean serum concentrations of biologically active isoniazid of the "slow" and "rapid" inactivators in the European-descent and Japanese-descent groups.

study in this laboratory. Nevertheless, the wide difference in the concentrations of biologically active isoniazid attained in the blood of the members of the two groups suggests that the manner in which an individual metabolizes isoniazid is determined by genetic factors. Support for this is gained by the fact that the European-descent group is approxi-

mately evenly divided between those with relatively high serum concentrations and those with low concentrations[3, 5-7]; few individuals have intermediate values. This distribution does not suggest the bell-shaped curve of biologic variation, but rather suggests a discontinuous system such as is frequently seen in the segregation of mendelian genes. It is particularly interesting that this trait appears to occur with a higher incidence in one ethnic group than in another. Recent studies [10, 11] of the urinary excretion of β-aminoisobutyric acid in humans demonstrate a somewhat similar phenomenon. The urinary excretion of significant amounts of this amino acid by certain subjects and of insignificant amounts by others has been found to be genetically determined. A remarkably wide variation among ethnic groups has also been noted. Less than 10 per cent of Americans of European descent excrete this substance, whereas approximately 60 per cent of those of Asian descent do. Careful family studies will be required to clarify the role of genetic factors in isoniazid metabolism. If this variation is eventually proved to be genetic in origin, a very simple and useful method, analogous in this respect to the blood groups and phenylthiourea taste testing, will be provided for the study of human differences.

In addition, the demonstration of a high incidence of rapid inactivators among persons of Japanese descent may provide another means of investigating the significance of biologically active isoniazid serum concentrations in the treatment of tuberculosis. It has been suggested that the clinical efficacy of isoniazid in the treatment of pulmonary tuberculosis varies in different subjects depending upon the pattern of metabolism of the drug and upon the maintenance of "adequate" concentrations of free drug in the blood. If the great majority of people of Japanese descent are rapid inactivators, as is the case in the small group reported here, then most tuberculous patients of Japanese descent would be expected to have serum concentrations of free isoniazid below the "optimal" range. One might expect, therefore, a significantly inferior clinical and bacteriologic response in such patients with tuberculosis treated on drug regimens containing isoniazid in conventional doses (300 mg. daily) than in other Americans treated on similar regimens. Although such data on non-Japanese descendants are readily available for comparison, we are unable to find reports of the results of isoniazid treatment of patients of Japanese descent. A retrospective analysis of the results of the treatment of such patients in the United States would be of considerable interest.

Microbiology Research Laboratory
Veterans Administration Hospital, and
Department of Medicine
University of Utah College of Medicine
Salt Lake City, Utah

H. Wm. Harris
Ralph A. Knight
Merle J. Selin

July 11, 1958

[10] DeGrouchy, J., and Sutton, H. E.: Am. J. Human Genet., 1957, *9*, 76.
[11] Gartler, S. M., *et al.*: Am. J. Human Genet., 1957, *9*, 200.

Correspondence

ON THE MECHANISM OF INCREASING THE BACTERIOTROPIC POTENCIES OF ISONIAZID IN THE PRESENCE OF PARA-AMINOBENZOIC ACID (PABA)

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

As reported by Urbančík,[1, 2] the bacteriotropic activity of isoniazid increases in the presence of appropriate amounts of *p*-aminobenzoic acid (PABA) *in vitro*. It was also shown by the same writer that a similar phenomenon occurs in either isoniazid-resistant or isoniazid-susceptible strains of *M. tuberculosis*.[2]

We have seen in our own experiments the same results and we tried therefore to discover the mechanism of this phenomenon.

It was shown by Duerr,[3] Johnson and Corte,[4] Bretonni,[5] and Mitchell and Bell[6] that simultaneous administration of isoniazid and PABA in either experimental animals or tuberculous patients results in increased blood concentrations of free active isoniazid if compared with therapy with isoniazid alone. It was supposed by these writers that this increase of isoniazid blood concentrations was attributable to competitive acetylation whereby the PABA is perhaps more and sooner acetylated than isoniazid. This effect of PABA is not specific; similar aromatic amines will give results varying only quantitatively from those achieved with PABA. On the other hand, it was shown by Martin and associates[7] that tuberculous guinea pigs do not have any, or have only a lowered, capacity to acetylate PABA, this being the result of metabolic disturbances which probably occur in the synthesis of coenzyme *A*.

Perhaps it is possible that such competitive acetylation may occur also in the *in vitro* environment. But in our opinion this hypothesis cannot explain the fact that the effect of isoniazid is increased also in the case of isoniazid-resistant strains of *M. tuberculosis*. We therefore tried to find another explanation for the "synergism" between isoniazid and PABA.

At first we tried to study the growth of either the H37Rv or the H37Rv-isoniazid-resistant strain of *M. tuberculosis* (kindly supplied by Dr. W. Steenken, Jr. from Trudeau Laboratory) on the surface of the Long liquid medium with and without PABA (figures 1 and 2). It may be seen that PABA in the concentration of 200 γ per ml. does not increase the definitive bacterial mass after four weeks of incubation, but that it increases the bacterial mass in early phases of the experiment, thus accelerating the multiplication of both strains.

Further experiments were performed to determine the endogenous respiration of the H37Rv strain in the presence and absence of PABA and isoniazid (by Warburg apparatus). As shown in figure 3, isoniazid increased the respiration while inhibiting the growth; and PABA decreased the respiration while stimulating the growth of this strain.

[1] Urbančík, R.: Rozhl. Tuberk., 1956, *16*, 487.

[2] Urbančík, R.: Am. Rev. Tuberc., 1957, *76*, 706.

[3] Duerr, E. L.: Am. Rev. Tuberc., 1957, *75*, 506.

[4] Johnson W., and Corte, G.: Proc. Soc. Exper. Biol. & Med., 1956, *92*, 446.

[5] Brettoni, B.: Minerva med., 1952, *2*, 674.

[6] Mitchell, R. S., and Bell, J. C.: Tr. Sixteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957, p. 105.

[7] Martin, S. P., *et al.*: Ciba Foundation Symposium on Experimental Tuberculosis: Bacillus and Host, Little Brown & Co., Boston, 1955, p. 102.

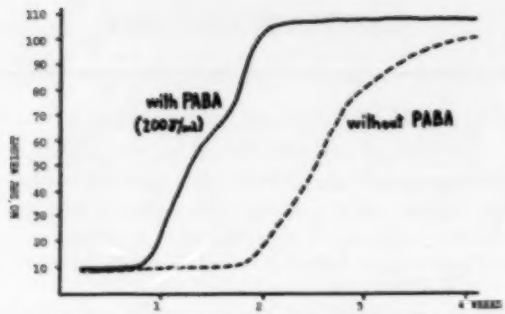


FIG. 1. Growth of strain H37Rv with and without PABA.

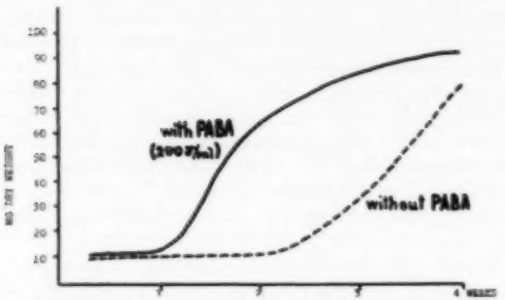


FIG. 2. Growth of strain H37Rv, isoniazid-resistant, with and without PABA.

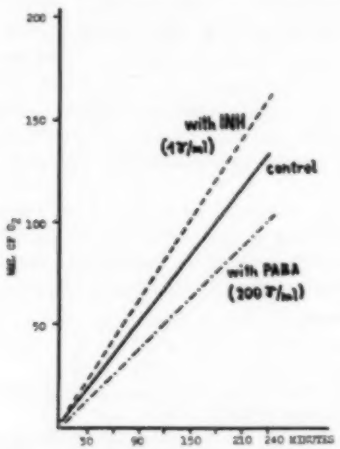


FIG. 3. Respiration of H37Rv strain in the presence of isoniazid (INH) and PABA.

These observations may be explained as compensatory mechanisms in which the influence of isoniazid results in growth inhibition accompanied by increased respiration, and vice versa for PABA. It is further known that "dormant" cells in general are more resistant to the influence of different agents. For *M. tuberculosis* this was clearly demonstrated by Hobby and Lenert[8] and by Mackaness and Smith.[9] The latter writers showed that in the presence of oxytetracycline, a rather bacteriostatic substance, isoniazid loses much of its tuberculotropic activity. It was shown by Urbančik[10] that, for the H37Ra strain, isoniazid, while not active in the presence of oxytetracycline, "reactivates" when PABA is added to the medium.

Thus, it seems to us that, beside the competitive-acetylation hypothesis, it is probable that growth acceleration induced by PABA results in increased susceptibility of bacterial cells to isoniazid regardless of former susceptibility or resistance to this drug.

However, much work must be done before this problem, perhaps of importance for practical chemotherapy, will be resolved. For instance, it is not clear in view of this hypothesis why sulfanilamide increases *in vitro* the tuberculostatic potencies of isoniazid on *M. tuberculosis*, as reported by Gillissen.[11]

Furthermore, our respiration experiments show some interference between the action of isoniazid and PABA. This deserves further investigation, which is now in progress.

Very sincerely yours

EUGEN KUBALA

TUBERCULOSIS RESEARCH INSTITUTE
PALISÁDY 47, BRATISLAVA I
CZECHOSLOVAKIA

April 26, 1958

Present address: Iskernikova 2, Bratislava XI, Czechoslovakia

[8] HOBBY, G. L., AND LENERT, T. F.: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 256.

[9] MACKANESS, G. B., AND SMITH, N.: Am. Rev. Tuberc., 1953, *67*, 322.

[10] URBANČÍK, R.: Rozhl. Tuberk (in press).

[11] GILLISSEN, G.: Ztschr. Hyg., 1953, *137*.

Books

THE CHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS, Third edition. By Esmond R. Long. Pp. 450, The Williams & Wilkins Company, Baltimore, 1958. Price $12.00.

No more appropriate authority could be found today to write a book on the chemistry and chemotherapy of tuberculosis than Esmond Long. For more than thirty-five years he has been associated with the accumulation of knowledge concerning this subject, both as a co-author with H. Gideon Wells and as an original investigator. No better understanding of the history of our knowledge in this field can be obtained than by studying comparatively the three editions of this book which have been published since 1923. Dr. Long, in this third edition, has charted that progress and brought together in critical fashion and in nice style all of the results of investigation on the chemistry of tubercle bacilli, the chemical changes in the tuberculous host, and the chemical aspects of contemporary chemotherapy of tuberculosis. This third edition is an entirely rewritten book, and the bibliographies are remarkably comprehensive and exhaustive.

Every investigator in the field of tuberculosis and every academic medical science library should have the book readily available.

It is inevitable, of course, that any reviewer working in a special field can find things to criticize in someone else's book in the same field. This reviewer finds a deficiency in Dr. Long's treatment of the general problems of chemotherapy, for example, in one respect: he failed to provide any discussion of the mechanisms of drug-treatment failure. Also, it seems to this reviewer that the author could have been a little less careless in his use of the terms "bacteriostatic" and "tuberculostatic" when applied to drugs such as streptomycin and isoniazid which have such powerful sterilizing (bactericidal) activity both *in vitro* and *in vivo* under conditions permitting bacterial multiplication. And, the author has omitted reference to studies which have established an important relationship between certain aspects of the bacteriologic response to isoniazid therapy and the biochemical disposition of that drug by the individual patient. However, for a book of such dimensions, these omissions or commissions are surely minor. Dr. Long modestly and wisely concludes his scholarly book with the statement that "Much is still to be learned, however, to furnish a satisfactory picture of the interlocking of applied therapy and physiologic and pathologic changes in the body."

GARDNER MIDDLEBROOK
Denver, Colorado

TUBERCULOSIS IN WHITE AND NEGRO CHILDREN. Vol. I. The Roentgenologic Aspects of the Harriet Lane Study. By Janet B. Hardy. Pp. 122, Harvard University Press, Cambridge, 1958. Price, $7.50.

TUBERCULOSIS IN WHITE AND NEGRO CHILDREN. Vol. II. The Epidemiologic Aspects of the Harriet Lane Study. By Miriam E. Brailey. Pp. 103, Harvard University Press, Cambridge, 1958. Price $4.50.

These two excellent volumes are based on the experience of the Harriet Lane Tuberculosis Clinic of the Johns Hopkins Hospital. In operation from 1928 to 1950, this clinic ad-

mitted for study and follow-up observation tuberculin-positive children less than two years old and their older siblings, as well as tuberculin-negative children regularly exposed to adults with infectious sputum in the home. Dr. Janet Hardy, Director of the Clinic from 1944 to 1949, introduces volume I on "Roentgenologic Aspects" with a short, useful chapter on definition of terms used in describing tuberculous lesions in children; and other chapters on segmental anatomy and roentgenographic techniques used in children. A hundred pages of roentgenographic reproductions follow, two to four on a page, with a paragraph of clinical history and comments on each film. The reproductions, drawn largely from the files of the Clinic, illustrate both normal findings in children's chest roentgenograms (enlarged thymus, hair shadow, azygos lobe); and the evolution over a period of months or years of such tuberculous processes as the primary complex healing with calcification, or progressing to excavation, or complicated by obstructive phenomena; extrapulmonary primary tuberculous lesions, hematogenous spread with subsequent calcification, miliary and cerebral lesions, tuberculous dactylitis, pleurisy, and pericarditis. The inclusion, where indicated, of bronchograms, photographs of autopsy specimens, and roentgenograms of patients with other lesions which may lead to errors in diagnosis (sarcoid, lymphosarcoma) broaden the scope of the book, which finishes with a section on the general indications for bronchoscopy and on the bronchographic technique used toward the end of the Harriet Lane Study.

Dr. Miriam Brailey, Director of the Harriet Lane Tuberculosis Clinic from 1932 to 1941, presents in volume II two separate epidemiologic analyses based on the 1,329 children included in the Harriet Lane data, the first on the prognosis of primary tuberculous infection in white and in Negro children, the second on the risk of developing chronic tuberculosis of the so-called "reinfection type" after initial infection in early childhood. From the earliest years of the study, thanks to the inspiration of Dr. Wade Hampton Frost, the data were gathered so that they could later be subjected to statistical analysis, but it is Dr. Brailey's lucid interpretation which brings them to life and makes them fascinating and delightful reading.

Out of the tremendous effort represented by this twenty-two-year study, and the welter of data which it furnished, these two authors have carved distinct entities, each according to her own interests. Volume I partakes of the nature of both a monograph and a textbook on tuberculosis in children; it is quite the best there is on the subject in the English language. It deserves to be studied by all physicians and medical students concerned with the clinical aspects of this disease in the very young. Volume II constitutes an important and basic contribution to our knowledge of the natural history of tuberculous infection in man. As such it will always be consulted by those who feel the need to know how tuberculosis behaved before the era of effective antituberculous drugs and by those who ponder the curious relationship between initial tuberculous infection in early life and the lesions of "reinfection" in young adulthood.

Some readers may regret the large format of these volumes; others may note wistfully that all roentgenographic reproductions in our country are so far inferior to the best in Europe; still others may question the use of the Foster-Carter nomenclature or the wisdom of including the chapter on bronchoscopy and bronchograms, or the interpretation of certain roentgenograms; but everyone will surely agree that the presentation and the style throughout are elegant, and that these books constitute a fine addition to medical literature.

MARGARET H. D. SMITH
New York, New York

CHRONIC ILLNESS IN THE UNITED STATES. Vol. I. Prevention of Chronic Illness. By the Commission on Chronic Illness. Pp. 338, Harvard University Press, Cambridge, 1957. Price, $6.00.

The basic issue faced in this volume is summarized by a statement from Dr. Leonard A. Scheele which is quoted in the introduction:

> The great obstacle to prevention is the development of methods for complete application of the knowledge and measures we have at present. The difficulties of application affect both research and practice.

This interesting book goes a long way toward developing a systematic approach to this problem by pooling the knowledge of recognized experts in the field of chronic disease.

This is the first of four volumes reporting the conclusions reached by the Commission On Chronic Illness which was established in 1949 to study the complex nationwide problem of chronic illness and which was dissolved in 1956 after they had "carried forward a program designed to accomplish the objectives of its founders." Throughout this period, Mr. Leondard W. Mayo was chairman of the commission.

Part I of this volume is entitled "Prevention: Its Role And Objectives." Prevention is divided into three phases: (*a*) promotion of health, which is defined as measures to avoid the occurrence of illness by encouraging achievement and maintenance of an optimal level of health throughout a person's life; (*b*) primary prevention, which deals with means for averting the occurrence of specific diseases; and (*c*) secondary prevention, which deals with halting the progression of a disease from its early unrecognized stage to a more severe one, and preventing complications or sequelae of disease.

It will be difficult for many readers to accept the rather unrealistic statements on promotion of health. One of these is the World Health Organization's definition of health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." A further example is the goal of health promotion stated by the President's Commission on the health needs of the nation:

> A steady glowing health is the goal. Cheerful people, full of vital strength and practicing the decorum of kindly behavior, accomplish more than grumblers and grouchers who snarl at each other.

The reviewer inclines toward Dr. Walsh McDermott's statement that:

> All living things, including man, are inevitably disease ridden and mortal; but that, within those immutable limits, some diseases are preferable to others. In the selection of which diseases we have, there is some element of choice, for the occurrence of particular diseases depends to a considerable degree on the forces we set up or tolerate in our particular society.

The chapter on primary prevention rests on sounder grounds, and accepts the fact that one can not always control certain hazards that are inherent in life.

The chapters on secondary prevention through periodic health examinations and through screening examinations give an excellent review of present knowledge in this field. It is pointed out that, as experience accumulates, it will be possible to pick out definite groups, based on age, physical characteristics, social status, and vocation, who will be shown to have a high incidence of certain diseases. Preventive examinations, done on

these groups, would therefore be expected to yield a high percentage of positive findings. This is a practical use of epidemiology as a tool in studying chronic disease.

Chapter six points out the critical shortage of personnel working in the field of chronic disease and stresses the importance of increased efforts towards training and recruitment in this field.

The chapter dealing with community planning for prevention rightly emphasizes the coordination of present facilities rather than the development of many different facilities working as isolated units. It makes the point that, "although seldom so regarded, a far-reaching instrument for coordination between hospitals and health departments exists in the hospital survey and construction (Hill-Burton) program." Another suggestion is that a guide be published to help communities select areas of activity on which to concentrate and to suggest sound ways in which to proceed. "Something is needed comparable to *The Control of Communicable Diseases in Man*."

Part II summarizes knowledge of prevention of selected chronic diseases and contributory factors. Each chapter is divided into etiology, primary prevention, and possible areas of research. In some of the chapters these subdivisions seem artificial and forced, but that is because there are at present wide gaps in knowledge in these important aspects. This method of organizing the information serves to make the gaps more obvious and should be a stimulus for future research.

An interesting appendix outlines the history of the commission and gives a list of its publications.

The volume is interestingly written, includes a comprehensive bibliography, and is very effectively indexed. It deserves a wide audience and justifies the commission's conviction that "full application of what is presented in this volume would so reduce the burden of chronic disease that the effects on the economic, social, and health status of the American people could be likened to the first great triumphs of preventive efforts against epidemic diseases."

HAROLD N. WILLARD
New York, New York

BOOKS RECEIVED

COCCIDIOIDOMYCOSIS. By Marshall J. Fiese. Pp. 253, Charles C Thomas, Springfield, Illinois, 1958. Price, $9.50.

NEUE TUBERKULOSTATIKA UND TUBERKULOSTATIKA-RESISTENZ VON TUBERKELBAKTERIEN. By A. M. Walter. Pp. 152, Georg Thieme Verlag, Stuttgart, 1958. Price, $4.70.

FUNKTIONELLE RÖNTGENDIAGNOSTIK DES MEDIASTINUMS AM BEISPIEL DES BRONCHIALKARZINOMS DEMONSTRIERT. By R. Karus. Pp. 55, Georg Thieme Verlag, Stuttgart, 1958. Price, $2.00.

THE ADVANCEMENT OF MEDICAL RESEARCH AND EDUCATION. From the Department of Health, Education, and Welfare, Washington, June 27, 1958. Pp. 81.

HANDBUCH DER TUBERKULOSE. Vol. I. Allgemeine Grundlagen. Edited by J. Hein, H. Kleinschmidt, and E. Uehlinger. Pp. 832, Georg Thieme Verlag, Stuttgart, 1958, Price, $42.40.

SCIENTIFIC COMMUNICATIONS RESEARCH DEPARTMENT. Vol. VII, July 1956–January 1958. Published by Brocades-Stheeman & Pharmacia, Amsterdam, 1958. Pp. 355.

THE AUTHOR PUBLISHER PRINTER COMPLEX, Third edition. By Robert S. Gill. Pp. 134, The Williams & Wilkins Company, Baltimore, 1958. Price, $2.25.

HORMONE PRODUCTION IN ENDOCRINE TUMOURS. Edited by G. E. W. Wolstenholme and Maeve O'Connor. Pp. 351, Little, Brown and Company, Boston, 1958. Price, $9.00.

MAJOR SURGERY FOR PULMONARY TUBERCULOSIS. By R. S. Francis and M. P. Curwen. Pp. 96, Macgibbon & Kee, London, 1958. Price, 12/6.

CHRONIC BRONCHITIS, EMPHYSEMA AND COR PULMONALE. By Stuart-Harris and Hanley. Pp. 245, John Wright & Sons Ltd, Bristol, 1957. (The Williams & Wilkins Co., Baltimore, exclusive U. S. agents.) Price, $8.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

The Award of the Trudeau Medal for 1958

The Trudeau Medal for 1958 was awarded to Dr. H. Corwin Hinshaw at the annual meeting of the National Tuberculosis Association, held in Philadelphia, Pennsylvania, May 18–23, 1958. The presentation was made by Dr. David T. Smith.

Dr. SMITH: The winner of the Trudeau Medal for 1958 is one of the few recipients of this award who has not had a personal battle with the tubercle bacillus. The years devoted to the study of tuberculosis were for the benefit of his fellow man and, hence, by definition, pure altruism.

The recipient's family was of Quaker stock which was distributed widely through Pennsylvania, North Carolina, Iowa, Idaho, and California. His parents were living in Eastern North Carolina before his birth but contracted malaria and decided to move to Iowa, where the recipient was born in 1902. His interest in experimentation was evident even as a small boy, when he was constantly tinkering with his toys and other mechanical devices. A member of the family reports that these early experiments were usually unsuccessful.

His formal education began to take shape when he received his AB degree from the College of Idaho in 1923. His broad interest in living things was developed in college as a hobby. He tramped the fields and woods, studying everything from birds to fungi.

He spent the year following graduation at the University of Pittsburg, trying to breed a strain of rabbits for genetic studies, but the rabbits refused to cooperate. His luck seems to have changed when he married Dorothy Youmans on August 6, 1924. The young couple moved to Berkeley, California, where Dorothy worked as a technician in zoology, and the recipient earned an M.A. degree in 1926 and a Ph.D. degree in 1927. The major work was in protozoology, with minors in biochemistry and bacteriology. His thesis was entitled "Protozoa of the Human Mouth" and his source material was the mouths of the prisoners in the San Quentin Prison.

Dr. Hinshaw's hobbies during this period were photography and a study of the blood-sucking insects found in the nests of wood rats in the deserts of Southern California. Both he and Dorothy were aware that many of the fleas in these rat nests were infected with the bacillus of bubonic plague. Dorothy accompanied him on these expeditions, but insists that she was not an active investigator but functioned as a control. Fortunately for our story, both escaped infection.

The recipient's thesis on the protozoa of the human mouth established his reputation as a protozoologist, and he was promptly given a permanent position on the faculty at the University of California. After one year in his position he resigned to take a position as Adjunct Professor of Parasitology of the School of Medicine, American University of Beirut in Syria. During the next three years he found that there were more and better human parasites in Beirut than in Berkeley. But a study of parasitology necessitates the study of the host as well as the parasite, so he decided to obtain his medical degree. From 1931 to 1933 he was a medical student at the University of Pennsylvania, but paid his expenses by working as an instructor in bacteriology in that medical school. In May, 1933, he left Philadelphia to become a Fellow in Medicine at the Mayo Foundation at

Rochester, Minnesota. In May, 1958, exactly twenty-five years later, he returns to Philadelphia to receive the Trudeau Medal for distinguished research in tuberculosis.

The recipient's contributions to protozoology and to various phases of medicine have been extensive and of a high quality. He is the author or co-author of more than 150 scientific papers and two books. The second book, entitled *Diseases of the Chest*, was published in 1955.

The story of the brilliant series of investigations which earned him the Trudeau Medal began in 1939, or nineteen years ago.

The discovery of sulfanilamide in 1936 was the first major breakthrough in the chemotherapy of bacterial diseases. It was natural and inevitable that sulfanilamide should be tried as a treatment for tuberculosis. Unfortunately, sulfanilamide was not effective in therapeutically attainable doses. This failure confirmed in the minds of nearly every one the conclusion drawn by Trudeau forty years previously, that tubercle bacilli cheerfully survive chemical agents which kill the hosts.

The recipient, almost alone, held tenaciously to the belief that sometime, somewhere a chemical agent would be found which could kill the tubercle bacillus without killing the host.

The pneumococcus resembled the tubercle bacillus in resisting sulfanilamide, but in 1939 some English investigators discovered that a new drug called sulfapyridine was very effective in killing the pneumococcus. The recipient suggested to his colleague, Dr. William H. Feldman, at the Mayo Clinic that sulfapyridine be tried in experimental tuberculosis in guinea pigs. This new drug failed also.

In 1940 the recipient learned of a newly synthesized sulfone compound called Promin® and obtained a supply ostensibly for clinical trials in pneumonia. Actually, he and Feldman diverted a part of this initial supply of Promin for a tuberculosis experiment. The initial experiment started in May, 1940, and five and one-half months later it was obvious that this drug could kill the tubercle bacilli in guinea pigs without killing the animal. Between 1940 and 1945, the experiments were repeated with this and other related compounds using every conceivable type of control. The results were conclusive. The guinea pig, which is much more susceptible to tuberculosis than man, could be cured with these chemicals. It is ironic that man could not tolerate the new drugs as well as the guinea pig, so Promin and its derivatives were not destined to be successful in the treatment of tuberculosis in man but were later found to be reasonably effective against leprosy in man.

Streptomycin was discovered by Waksman in 1944 while he was searching for an antibiotic to kill the colon bacillus. It is purely a biologic accident that streptomycin also kills, or at least effectively inhibits, the tubercle bacillus. The recipient and Feldman, with their precise experimental methods, succeeded in a few months in demonstrating the effectiveness of streptomycin in tuberculosis in both guinea pigs and man.

As soon as the type of experimental work and the name of William H. Feldman were mentioned, I am sure that all of you knew that the name of the Trudeau Medalist for 1958, was H. Corwin Hinshaw.

Dr. Hinshaw: Dr. David Smith's reputation as a skillful story teller has been sustained tonight. He has that rare ability to make the truth sound like glamorous fiction, and he can make utter fiction sound like gospel truth!

I am a very fortunate person to be here tonight. Many good fortunes have been mine. My greatest stroke of luck was when I met and married Dorothy Youmans, my bride of thirty-three years, who is here tonight to share this gratifying experience.

My meeting with Dr. William Feldman was the most fortunate single event in my research career. He is a scholar, a gentleman, and a scientist of meticulous integrity with

an amazing breadth of vision. Actually, what he and I were able to do in tuberculosis research was well recognized when the Trudeau award was made to him in 1955.

I was fortunate to choose this city (Philadelphia) and the University of Pennsylvania in which to secure my clinical training. (I received my M.D. here exactly twenty-five years ago; in 1933.) I am grateful to the Mayo Clinic for further training and sixteen years of rich experience.

It was here in Philadelphia, in this hotel and in this very auditorium, where Bill Feldman and I gave our first paper—before any national society—reporting successful chemotherapy of tuberculosis; experimental and clinical. This was sixteen years ago—in 1942— when we talked freely (at least to one another) of the *possibility* of successful chemotherapy of tuberculosis which is now a reality. Few men have lived to see such dreams become truth on such a vast scale in such a short period.

I see many faces in front of me tonight of men who have participated as leaders of these exciting projects during the past sixteen years—several who deserve the Trudeau Medal. And I see others, younger and equally diligent—equally inspired—who may some day be in this fortunate position. To them I give this advice: Choose the right institution in which to work, choose the right collaborators with whom to work and to dream, and—marry the right girl!

ATS Formula for Determining Irregular Discharge Rates*

At its meeting in the fall of 1957, the Program Activities Committee of the ATS asked Dr. R. H. Browning, Director of the Ohio Tuberculosis Hospital, Columbus, to proceed with the development of a standard formula for determining irregular discharge rates. He has pursued this assignment diligently, and the following result has the approval of the Program Activities Committee, the Executive Committee, and innumerable individuals who were kind enough to review it and make suggestions.

As in all statistical studies, the terms must be defined, and strict classifications observed so that reports from different institutions will be comparable. This formula is an effort to accomplish these purposes. Its use will help all hospitals to determine whether their irregular discharge rates are actually high or low, in comparison with rates of other institutions.

Obviously, the deduction of certain types of discharges from the number to be considered will result in higher percentage figures in the irregular discharge category. It is hoped that this formula will be widely adopted and used so that statistics in this regard will be comparable.

1. *Total* of discharged patients (dates) _____
2. Total deductions _____
   a. Deaths _____
   b. Non-TB discharges _____
   c. Transfers to other hospitals for continuation of TB treatment _____
   d. Discharges of patients admitted only for observation, diagnostic procedures, special examinations or follow-up, and for whom less than 30 days hospitalization was anticipated by original intent. _____
   e. Discharges of court-order patients from locked wards _____

* The Veterans Administration is currently employing the identical formula for its statistical purposes.

3. Net TB discharges _____
4. Total irregular discharges _____
   a. AMA† _____
   b. AWOL‡ _____
   c. Disciplinary§ _____
5. Per cent irregular discharges (Item 4 ÷ 3 × 100) _____

† AMA—Patient announces his intentions to leave, completes the routine administrative and medical checkouts, and signs a hospital release form.

‡ AWOL—Patient absconds without notice or fails to return from an authorized period of absence.

§ Disciplinary Discharge through administrative action, usually for violation of rules and regulations.

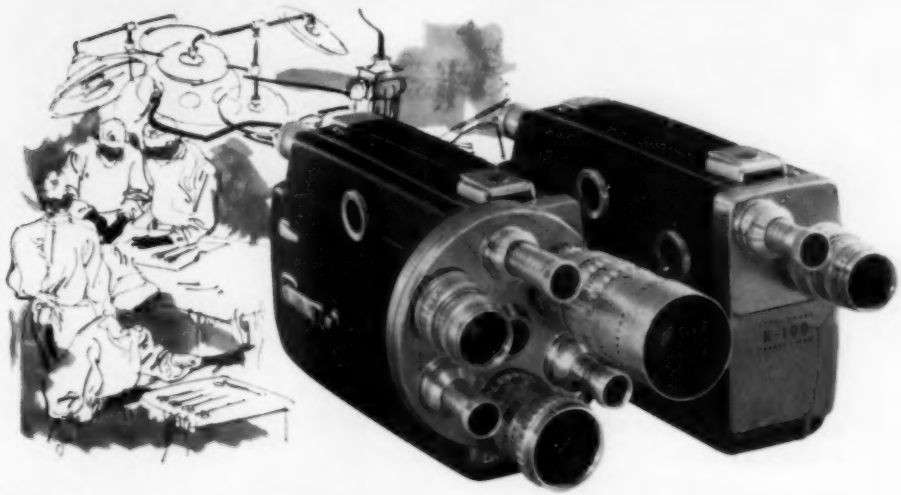
NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and and approved:

1959—Chicago, Illinois—May 24-29
1960—Los Angeles, California—May 15-20

16mm Cameras... not only the finest to work with

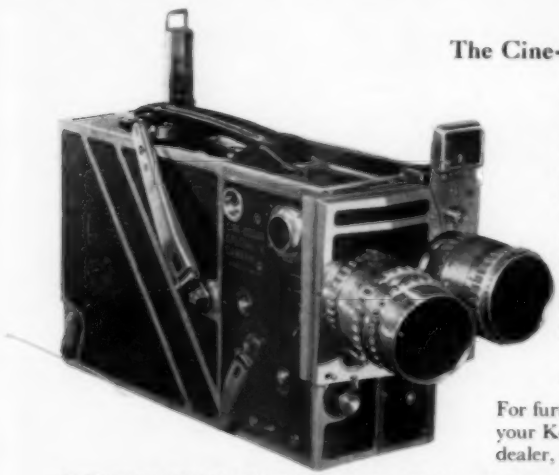
... but the least expensive to maintain



The Cine-Kodak K-100 Cameras . . .

Superb optics . . . precision engineering . . . distinguish not only the work produced with this fine camera but help assure long life and trouble-free performance.

Special features include a pre-stressed spring motor for 40-foot film run on single winding, full speed range—16 (standard speed) to 64 (slow motion) frames per second, simplified roll-film loading. Single-lens model priced from $299; turret model, from $337.



The Cine-Kodak Special II Camera

With available auxiliary equipment and full range of Kodak Cine Ektar Lenses, this Cine-Kodak Special II embodies a complete 16mm motion-picture-taking system capable of coping with highly critical situations. Its reflex finder shows the exact field covered at all distances, establishes focus, eliminates parallax problems. A long-running spring motor with quickly interchangeable 100- and 200-foot film chambers helps get all the action. From $1,365.00.

Prices are list and subject to change without notice.

For further information, see your Kodak photographic dealer, or write:

EASTMAN KODAK COMPANY, *Medical Division*, Rochester 4, N. Y.

Kodak
TRADE MARK

EMERSON
Pleural Suction Pump

1. **Very high volume.**
   Aspirates 30 liters per minute at a setting of 10 centimeters of water.

2. **Low, constant pressure.**
   Does not climb appreciably even with tubes wholly obstructed.

Designed for continuous service. May be adapted for 2 patients at once with 2 tubes to each. * * Made by the makers of Emerson Respiration Assistors for IPPB, Controller-Assistors for anesthesia, and Chest Respirators for bronchoscopy.

Please write

J. H. EMERSON COMPANY
CAMBRIDGE 40, MASS., U. S. A.

EUREKA
DUPLISNAPS®

SPEED MAILINGS

SIMPLIFY MAILINGS

DUPLISNAP carbon interleaved Mail-Aids. Made in sets of 2, 3, 4, 5 and 6 sheets, 33 labels to each sheet. Interleaved with smudge resistant carbon. For use with typewriter.

EUREKA SPECIALTY PRINTING CO.
530 ELECTRIC STREET, SCRANTON 9, PA.

Vocational Rehabilitation of the Tuberculous

by Sol L. Warren, Ph.D.

193 pages, paper cover **$2.00**

This volume should be in every medical library and medical school as a reference text. Every physician working with tuberculosis patients will find this book exceedingly valuable.

Essentially, Dr. Warren's book describes the study he conducted to determine whether any differences could be found between a group of ex-tuberculosis sanatorium patients who, following discharge, participated in a state agency vocational rehabilitation program, and a group which did not participate.

His findings disclosed that patients who participated in the rehabilitation program exhibited postdischarge histories which were distinctly different from those of the patients who had not participated, the differences being virtually always in favor of the former.

Your order can be filled promptly.

NATIONAL TUBERCULOSIS ASSOCIATION

1790 Broadway **New York 19, N. Y.**

3
essential drugs
for chemotherapy
of
tuberculosis

NYDRAZID
Squibb Isoniazid

The most potent anti-tuberculosis agent available—developed in Squibb laboratories.

Tablets, 50 and 100 mg. (scored), bottles of 100 and 1000
Syrup, 10 mg. per ml., pint bottles
Injection, 100 mg. per ml., 10 ml. vials

DISTRYCIN
Squibb Streptoduocin

As active as streptomycin or dihydrostreptomycin, but with markedly less toxicity.

Powder, vials containing 1 Gm. and 5 Gm.
Solution, 0.5 Gm. per ml., vials of 2 ml. and 10 ml.
0.5 Gm. per 1.25 ml., vials of 2.5 ml. and 12.5 ml.

REZIPAS
Squibb PAS Resin

Many patients unable to tolerate PAS can take Rezipas.

1 pound jars

SQUIBB

A PIONEER IN ANTI-TUBERCULOSIS RESEARCH

for depression

▲Deprol▲†

Clinically confirmed in over 2,500 documented case histories[1,2]

CONFIRMED EFFICACY

Deprol ► acts promptly to control depression *without* stimulation

► restores natural sleep

► reduces depressive rumination and crying

DOCUMENTED SAFETY

Deprol is unlike amine-oxidase inhibitors

► does not adversely affect blood pressure or sexual function

► causes no excessive elation

► produces no liver toxicity

► does not interfere with other drug therapies

Deprol is unlike central nervous stimulants

► does not cause insomnia

► produces no amphetamine-like jitteriness

► does not depress appetite

► has no depression-producing aftereffects

► can be used freely in hypertension and in unstable personalities

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

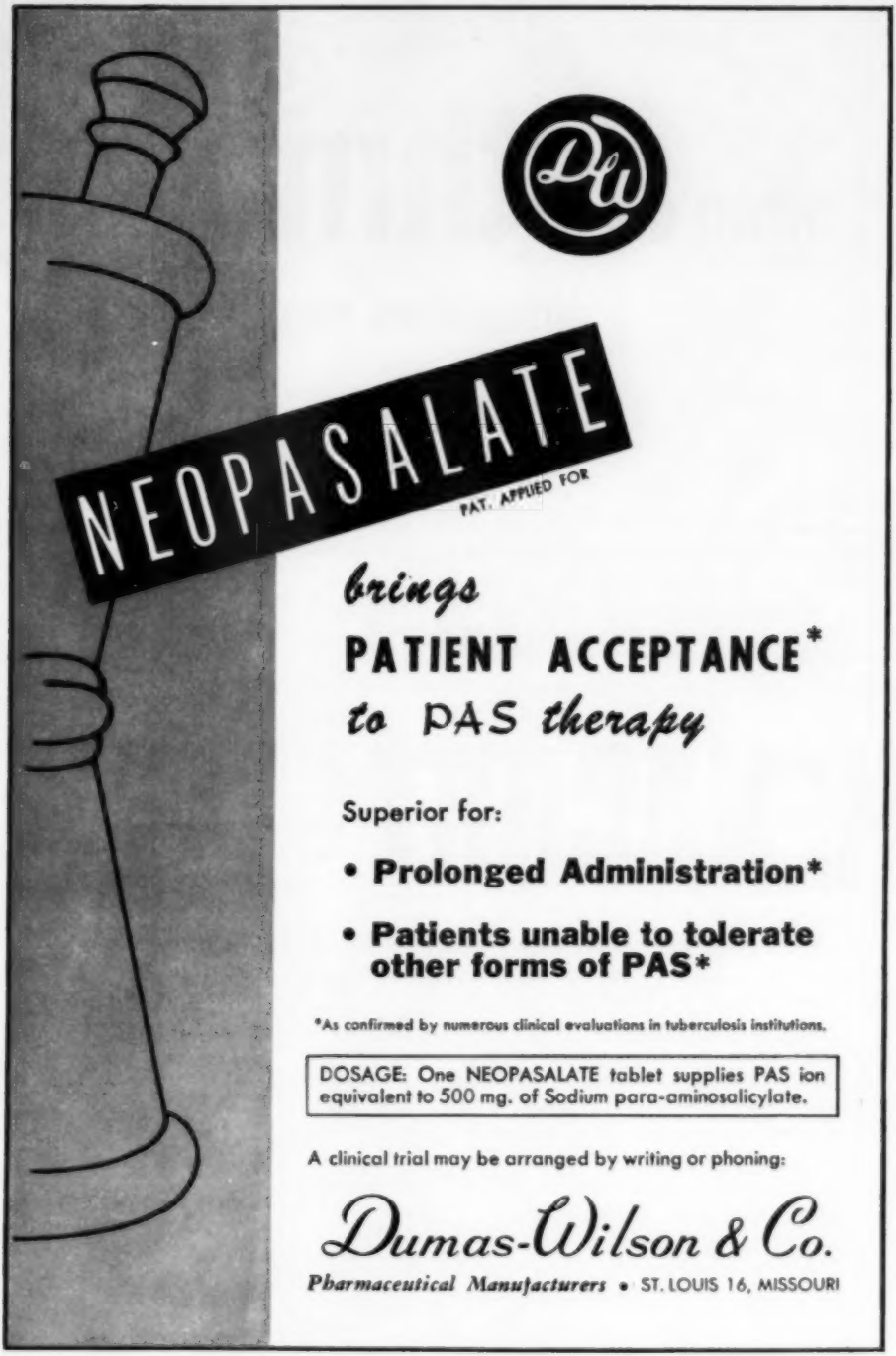
Composition: Each tablet contains 400 mg. meprobamate and 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl).

Supplied: Bottles of 50 scored tablets.

1. Alexander, L.: Chemotherapy of depression—Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. J.A.M.A. 166:1019, March 1, 1958. 2. Current personal communications; in the files of Wallace Laboratories.

†Trade mark CO-7001

Literature and samples on request WALLACE LABORATORIES, *New Brunswick, N. J.*


NEOPASALATE
PAT. APPLIED FOR
brings
PATIENT ACCEPTANCE*
to PAS therapy
Superior for:
• Prolonged Administration*
• Patients unable to tolerate other forms of PAS*
*As confirmed by numerous clinical evaluations in tuberculosis institutions.
DOSAGE: One NEOPASALATE tablet supplies PAS ion equivalent to 500 mg. of Sodium para-aminosalicylate.
A clinical trial may be arranged by writing or phoning:
Dumas-Wilson & Co.
Pharmaceutical Manufacturers • ST. LOUIS 16, MISSOURI

when asthma

diminishes ventilation

Kenacort therapy improves ventilation and increases vital capacity.[1] Dyspnea and bronchospasm are generally relieved within 48 hours and sibilant rales often disappear. As coughing and wheezing diminish your asthmatic patient will sleep more comfortably.

Kenacort possesses potent antiallergic and anti-inflammatory properties. This means that the benefits of improved pulmonary function and subjective well-being may be sustained on lower maintenance dosage than is possible with other steroids.

Kenacort is indicated in the treatment of asthma, allergies, arthritis and dermatoses —

KENACORT
Squibb Triamcinolone

with far less gastric disturbance [1,2,5,9]
without salt and water retention [2,6]
with no potassium loss [4,9]
without unnatural psychic stimulation [1,2,5]
with no adverse effect on blood pressure [1,2,4,5,7]
on a lower daily dosage range [1,5,7]

Supply: Scored tablets of 1 mg. and 2 mg.—Bottles of 50. Scored tablets of 4 mg.—Bottles of 30 and 100.

1. Sherwood, H., and Cooke, R.A.: J. Allergy *28*:97 (March) 1957 2. Hartung, E.F.: J.A.M.A. *167*:973 (June 21) 1958. 3. Boland, E.W.: Geriatrics *13*:190 (March) 1958. 4. Hellman, L., and others: A.M.A. J. Dis. Child. *94*:437 (Oct.) 1957. 5. Shelley, W.B.; Harun, J.S., and Pillsbury, D.M.: J.A.M.A. *167*:959 (June 21) 1958. 6. Feinberg, S.M.; Feinberg, A.R., and Fisherman, E.W.: J.A.M.A. *167*:58 (May 3) 1958. 7. Friedlaender, S., and Friedlaender, A.S.: Antibiotic Med. & Clin. Ther. *5*:315 (May) 1958. 8. Dubois E.L.: J.A.M.A. *167*:1590 (July 26) 1958.

SQUIBB

Squibb Quality — the Priceless Ingredient

KENACORT IS A SQUIBB TRADEMARK

DIFCO

Antisera and Antigens

Enteropathogenic Coli Klebsiella Salmonella

Shigella Brucella Leptospira Streptococci

*

C Protein Antiserum and Standard

Infectious Mononucleosis Antigens and Standards

Potent *Stable* *Specific*

Descriptive literature available on request

Specify

DIFCO LABORATORY PRODUCTS

BIOLOGICS CULTURE MEDIA REAGENTS

POSITIONS AVAILABLE AND WANTED

RATE $3.50 per insertion for 25 words or less; additional words—15 cents each. Bold face semi-display $5.00 per insertion for 25 words or less; additional words—20 cents each. Box number—75 cents extra. Employment Bureau—$10.00 per column inch. Remittance should accompany copy and be sent to: American Review of Tuberculosis and Pulmonary Diseases, 280 Madison Ave., New York 16, N. Y.

WANTED: Physician interested in pulmonary diseases for 170 bed state supported tuberculosis hospital; established 3 years; active surgical program, outpatient service, x-ray and laboratory; consultants in all branches. U. S. citizenship and Alabama medical license or eligibility therefor required. Salary $8500 to $9500 depending on qualifications. Quarters to be made available. Apply Medical Director, Sixth District Tuberculosis Hospital, 800 St. Anthony Street, Mobile, Alabama.

WANTED: RESIDENTS IN PULMONARY DISEASES for July 1, 1959 in a 600 bed hospital in the Ozarks; fully certified; excellent facilities; research and teaching program; generous salary. Write: Charles A. Brasher, M. D., Superintendent and Medical Director, Missouri State Sanatorium, Mt. Vernon, Missouri.

PANRAY
ANTI-TUBERCULOUS DRUGS FOR

TARGET POINT CHEMOTHERAPY

"Target point" is that point in
the therapy of pulmonary tuberculosis
when the following
three conditions co-exist:"[1]

Bacteriologic remission
Absence of cavity
X-ray stability

THE Panray CORP. 266 S. DEAN STREET · ENGLEWOOD, N. J.

Sole Canadian Distributors: Winley-Morris Co., 6579 Somerled Ave., Montreal 29, P. Q.

Ref.: [1]V.A. Preamble to Protocols, 13th rev. 3/15/57.